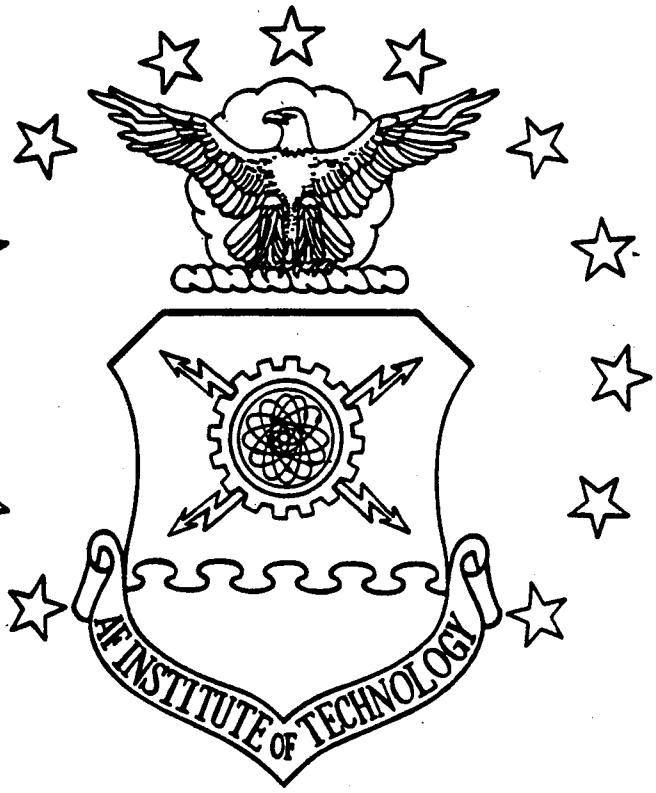

EXPERIMENTAL DEFINITION FOR IMPLANTATION OF THE AFIT CORTICAL MULTIELECTRODE ARRAY

THESIS

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AFIT/GE/ENG/94D-27

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THESIS

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of the Air Force Institute of Technology
Air University
In Partial Fulfillment of the
Requirements for the Degree of
Master of Science in Electrical Engineering

Adam G. Spenik, B.S.E.E.
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Abstract

A two-dimensional, multiplexed array of 256 electrodes (16 $\times$ 16) has been fabricated using conventional CMOS integrated circuit fabrication techniques. The size and spacing of the electrodes (160 $\times$ 160 μm squares with center-to-center spacing of 250 μm) approximate that of the cortical columns. The device is mounted on a small, biocompatible, chronically implantated package and a protocol to implant this package on the visual cortex of a ferret and a Rhesus monkey was developed and approved. An instrumentation setup to record the data from the device is described as well as the methods used to demultiplex the data. Testing of the device in a simulated cortical environment indicates that the device is capable of recording signals with amplitudes as low as 20 μV. This capability is significant as the level of the cortical signal ranges from 20–500 μV. Since each electrode is bidirectional, the device can also be used to stimulate the cortex. An experiment is envisioned in which the primary and secondary visual areas of a mammal are monitored simultaneously in an attempt to gather data while the brain is actually performing perception. This type of study will provide insight to the nature of the organization of the cortico-cortical connectivity matrix and its relation to the function of the cortical column system.

EXPERIMENTAL DEFINITION FOR IMPLANTATION OF THE AFIT CORTICAL MULTIELECTRODE ARRAY

I. Introduction

1.1 Justification

Recognition of a complex visual scene is a task that is very easily performed by a human or even an infra-human. Human and many lower animal visual systems have certain desirable capabilities. Primates especially have visual systems with high foveal acuity, color discrimination, and impressive form perception. If such capabilities could be duplicated by a machine, they would have immediate direct application in the automatic target acquisition and terminal guidance phases of smart missile systems and in many commercial and medical areas. Unfortunately, researchers have found that it is extremely difficult to build a machine capable of duplicating similar performance. No reliable automatic target locator or terminal guidance system based on visual form has ever been put into operational service even though research on this so called "pattern recognition" problem has been vigorously pursued for forty years. It is clear that there are some fundamental capabilities immanent in mammalian visual systems that scientists simply do not understand. If these naturally evolved techniques could be discovered from properly instrumented animal visual systems, it might be possible, given the current advanced state of electronic fabrication capabilities, to make a fundamental breakthrough in weapon guidance systems and commercial vision applications.

An understanding of how the brain performs perception and comprehension would have far-reaching effects in many other areas as well. For example, if one could model the visual perception system, incredible advances could be made in areas

ranging from security applications to improved weather prediction. Obviously, these areas are of great interest to the Department of Defense, and could be applied to commercial uses as well. Finally, it is possible that increased knowledge of the brain could lead to a new type of prosthetic device capable of directly stimulating the sensory perception centers in the brain.

1.2 Background

Twelve years have past since the implantation of the first AFIT multielectrode array [18]. This device, which consisted of sixteen multiplexed electrodes arranged in a four-by-four array, was implanted on the visual cortex of a laboratory beagle (Canis familiaris) in 1982. Electroencephalogram (EEG) data and visually evoked response (VER) data were collected. The measured EEG signal was approximately 0.5 mV to 1 mV. The device functioned for fifteen days before being removed and the subject "Ricky" recovered with no apparent side effects. Ricky was euthanized at the age of 13.5 years because of deteriorating heath caused by his advanced age. After death, a necropsy was performed to examine the multielectrode array implantation site. It was noted that the bone over the site had completely regenerated and had a somewhat wrinkled appearance. When the section of skull over the left occipital cerebral cortex was reflected back, a small section of what appeared to be cortical tissue approximately 0.5-1 mm in diameter adhered to the bone [25]. This tissue appeared to be healthy tissue and in fact, a histologic examination determined that the adhesion was an incidental finding and the condition of the tissue was consistent with conditions normally found in aged dogs [22]. Furthermore, the only difference between the cerebral cortex underlying the surgical site and the corresponding region on the other hemisphere was the presence of a pale brown globular pigment scattered within the superficial grey matter immediately below the fibrous adhesion [29]. This pigment was randomly distributed with a slightly increased presence near blood vessels and did not appear to have been attacked by the animal's immune system.

Also, the thickness of the grey matter and the form, structure, and number of nuclei appeared similar between both hemispheres. This experiment validated the concept of using multielectrode arrays for neural research; however, it also identified several improvements that were necessary. The improvements included the need for more electrodes, smaller electrodes to better match cortical columns, better fabrication processes to improve the electrical characteristics, methods to better protect the device, methods to improve the signal-to-noise ratio, a thorough definition of the experiment, and an improved surgical and implantation procedure. In the years since then, several thesis efforts have been made to improve the device design and to solve the problems encountered with the first implementation [1, 39, 37, 28, 33].

The current design of the AFIT multielectrode array is described in detail by Reid in his 1993 thesis [33]. This device consists of 256 electrodes laid out in a 16 x 16 array. A sketch of this design is shown in Figure 1, and a photograph of the unpackaged device is shown in Figure 2. The reference pad, which runs along two sides of the electrode array, has a direct connection from the chip and is used to provide a local reference voltage for the recorded cortical signal.

The array electrodes are 160 x 160 μm squares with a center-to-center spacing of 250 μm. Each electrode is individually accessesed for one clock cycle out of 256 by the multiplexing circuitry. An 8–bit counter is used to generate an 8–bit address which is used to specify each electrode. The counter is controlled by an external clock signal. The rate at which the counter increments is one–half the rate of the external clock signal. The counter can be set to a logical zero by the use of an external reset (active low) signal. This reset signal allows the system to be initialized to a known state.

The 8–bit output of the counter is split into two 4–bit signals and connected to two 4 to 16 decoders. The 16 outputs of the first decoder control transmission gates, which act as switches between the column I/O channels and the single multiplexed output channel. When one of the 16 decoder lines is selected, its' transmission gate

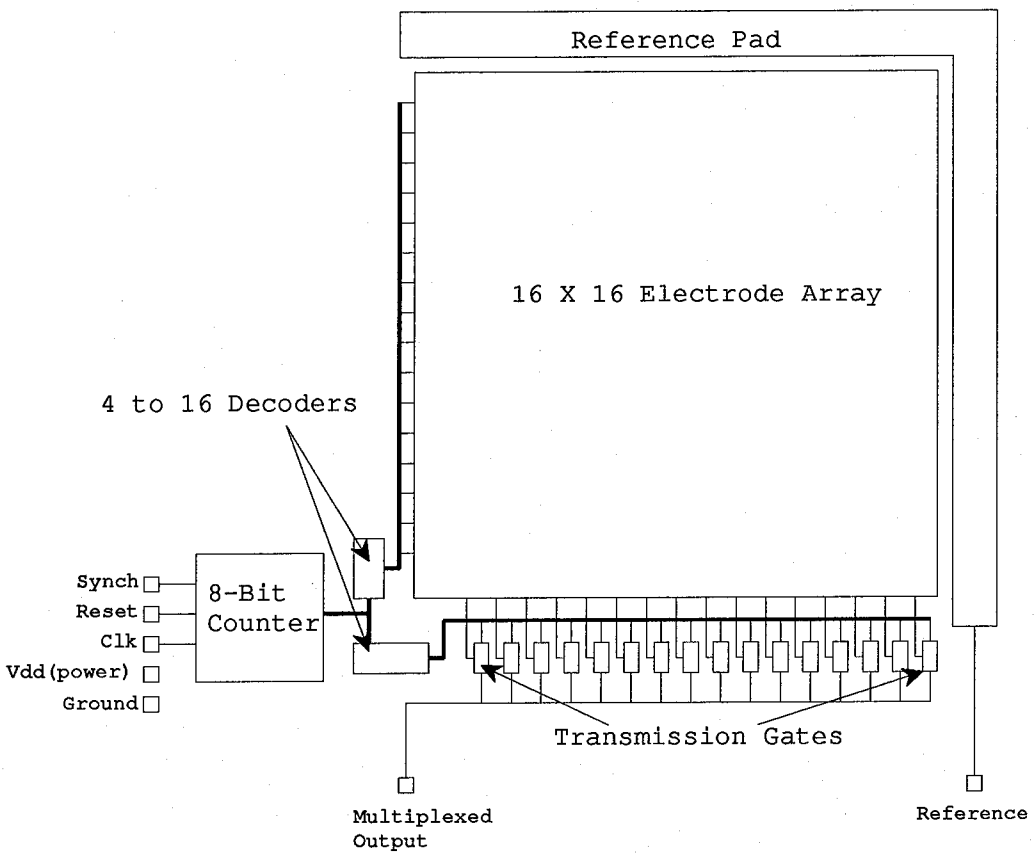


Figure 1. Sketch of the current AFIT multielectrode array [33].

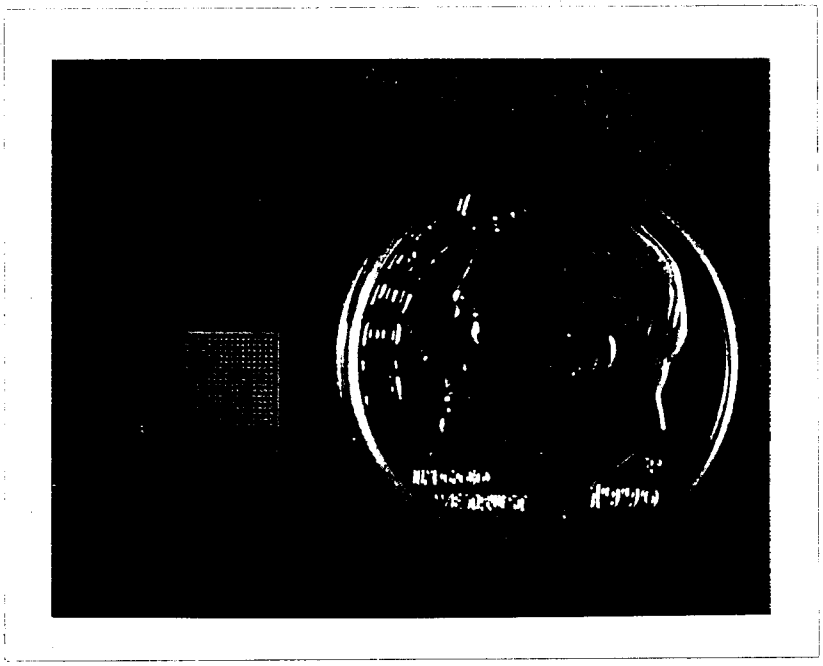


Figure 2. Photograph of the AFIT multielectrode array.

is enabled and the column I/O channel is connected to the multiplexed output. Note that the decoder only selects one output at a time. Therefore, only one of the column I/O channels will be connected to the multiplexed output channel at a time.

Similarly, the second decoder is used to select one of the 16 electrodes present in each column. Since the two decoders select only one I/O column and only one electrode in that column, only the signal from one electrode is present on the multiplexed output channel at any time. Electrode numbering is shown in Figure 3. When the reset signal is asserted (ground is applied to the reset input), electrode (1,1) is connected to the multiplexed output channel. Individual electrode impedance in a simulated brain environment was estimated to be 2.1 kΩ [33]. Average current flow of the device at 3.3 V was approximately 110 mA [35].

This final design has been tested and found to be functioning correctly [33, 34, 35]. Furthermore, operation in a simulated brain environment showed that the device should be expected to operate for at least 24 hours *in vivo*.

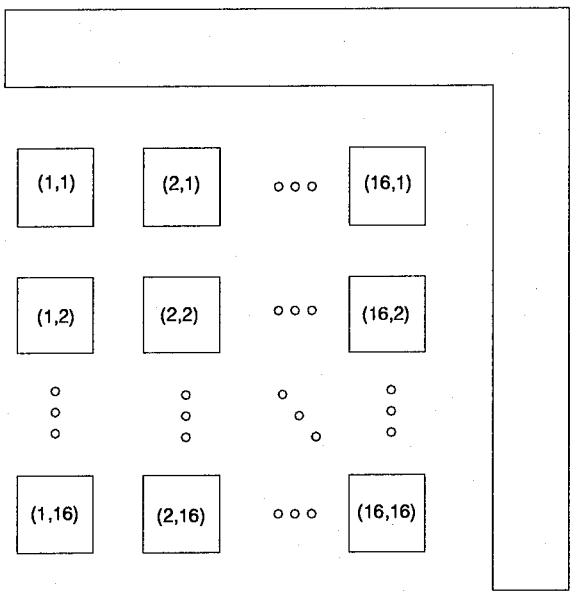


Figure 3. Electrode numbering scheme [33].

The completion of Reid's effort brought the AFIT array to the stage where an implantation experiment was possible. However, to successfully perform this implantation, many details still remained to be addressed, such as the definition of the experiment to be performed and the design of the instrumentation setup required to complete the study.

1.3 Scope

As previously mentioned, many open questions remained related to conducting a successful implantation. Therefore, this thesis effort is aimed at answering all of the open questions, designing the necessary instrumentation setup, receiving the required implantation approval, and performing the experiment.

At the start of this thesis effort, the AFIT multielectrode array had been successfully tested in a simulated brain–like environment. The next logical step was to proceed with the implantation. However, there was still much work to be done before the implantation experiment could be performed. The emphasis for the AFIT research up until now had been very device oriented. These efforts primarily fo-

cused on the improvement of the device; the design and optimization of the actual experiment had not yet been well defined. Also, to ensure that meaningful data were collected, a thorough understanding of the device operation and limitations as well as an understanding of the current knowledge of cortical physiology was necessary. Some of the questions that had to be answered are as follows:

- What is the nature of the signals to be expected on the surface of the cortex?
- Is the device capable of recording these signals?
- Can the device be used for stimulation, and if so, what input should be used?
- What species could be used as experimental subjects?
- What are the experiments which could be performed using this device?
- What is the instrumentation needed for the experiment?
- Once the data is collected, how should it be analyzed?
- What improvements can be made to the device?

This thesis effort addresses these questions.

1.4 Approach

The first phase of this effort was an evaluation of the current knowledge of cortical physiology and the capabilities of the AFIT array. Knowledge of how the brain operates has increased greatly since the first implantation. This knowledge was reviewed and evaluated to determine if the research approach being planned by AFIT was reasonable. Fortunately, the literature provided several examples demonstrating the usefulness of surface electrodes for the study of cortical processes. Additionally, the literature provided new theories on how the brain might perform visual recognition. Parts of these theories may be tested using the AFIT array.

The second major phase of this effort was to prepare for and complete implantation. This effort included not only the processing and packaging of the devices, but

also the design and construction of the necessary test setup and the coordination of all required approval requests. A protocol describing in intricate detail the intended research is required for any research use of live animals. First, this protocol is reviewed by the Armstrong Laboratory Animal Care and Use Committee (ACUC) to assure that the research is in full compliance with pertinent DoD regulations, policies, and federal statutes. Next, since this study involves the use of a non–human primate, the animal use protocol must be submitted to the Air Force Non–Human Primate Review Committee at Brooks AFB for further review. Finally, the protocol is submitted to the Office of the Air Force Surgeon General for final approval. Obviously, the intended study cannot be performed without the appropriate approval. Therefore, the development of an acceptable protocol and its' approval was a a major goal of this thesis effort. The animal use protocol submitted as a request for approval of the AFIT study is included as Appendix B.

Much of the first phase was completed concurrently with the extensive post-processing steps that had to be performed on the chips to protect them in the saline environment of the brain. A thorough review of all the details related to the planned implantation was made, including the performance of surgical dry runs executed by Dr Cooper, DVM. A significant objective of the dry runs was to test the fit of the implantation package designed by Reid [33]. Reid's design of the implantable package was intended to allow chips to be replaced in a subject without additional surgery. The package consisted of a hollow mount that would be chronically implanted in the skull. The multielectrode array was mounted on a header package and fitted inside a hollow cylinder which in turn fitted into the center of the mount. If a chip were to fail while in a test subject, it could be relatively easily removed and replaced with another. Unfortunately, from this review it was determined that since an opening to the interior of the skull was maintained, Reid's design of the implantation package carried with it a large risk of infection. Therefore the implantation package was redesigned using a similar approach to that used in the first implantation [26].

The design of the equipment setup was intended to circumvent the problems encountered with the first implantation [5]. In the first implantation experiment the data collection process was very cumbersome and did not lend itself to real time evaluation or subsequent easy analysis. In fact, the data analysis was the subject of two follow-on thesis efforts [5, 17].

1.5 Order of Presentation

Chapter 2 provides a review of the current knowledge of how the brain functions and the techniques used by other researchers to study the brain. Based on the current knowledge, several potential experiments using the AFIT multielectrode array are proposed. Chapter 3 discusses the methods employed to accomplish the stated goals of this thesis effort. A conclusion and suggestions for future work are provided in Chapter 4. For those readers unfamiliar with cortical physiology, Appendix A presents a more basic biological background review of the operation of neurons, the brain, and the visual processing system. Next, the final version of the animal use protocol is included as Appendix B. Finally, the steps needed to prepare and package the chips are outlined in Appendix C.

II. Literature Review

2.1 Introduction

As mentioned in Chapter 1, an understanding of the physiology of the cortex is necessary for the success of this effort. A brief overview of the basic characteristics of the brain and the visual perception system is given in Appendix A. This overview discusses several key concepts that warrant emphasis.

- Information is transmitted by a neuron by the generation of electrical impulses known as action potentials. These signals propagate down the cell's axon and are converted to chemical signals at the synapses, which are the contact points between neurons.
- The generation and transmittal of action potentials affects the excitability of other neurons.
- The electric fields produced by the activity of neurons can be detected by electrodes resting on the surface of the cortical tissue and subsequently recorded.
- The neurons in the cortex are grouped functionally into cortical columns, which consist of several hundred to several thousand neurons functioning together to perform a given task. These cortical columns are believed to be the smallest functional elements in the cortex [26].
- There is virtually no transmission of data *across* the cortical sheet. The cortical columns are accessed primarily through input/output axons running through the cerebral alba and between the cortex and the brain stem.
- Analysis of the interconnecting matrix between cortical columns may provide new insight to the function of the brain [26].

The AFIT multielectrode array design was based on these key concepts. Obviously, much more information is needed for the successful use of the AFIT device. Therefore, an extensive review of the pertinent literature was conducted. The next two

sections discuss previous research and physiology related to the proposed research utilizing the AFIT multielectrode array.

2.2 *Previous Research*

Research similar to that being proposed by AFIT relies on the use of either intracortical probes or surface electrodes. Intracortical probes are designed to penetrate the cortical surface in order to record either the response of (or stimulate) *individual* neurons, or groups of cortical neurons lying substantially beneath the cortical surface. Surface electrodes, on the other hand, are used to record or stimulate the *group* of neurons directly beneath the electrode. The electric field measurements taken from the surface of the brain are called electroencephalogram (EEG) tracings. An EEG represents the averaged excitatory state of the group of neurons lying directly beneath the measuring electrode [15].

The first research using surface multielectrodes relied on the use of wire bundles placed on the surface of the cortex. In 1966 DeMott reported on the use of an electrode array covering an area about 7×4 mm and made up of 400 closely spaced (0.25 mm center-to-center) wires [7]. DeMott's results obtained from a variety of animals, demonstrated substantial differences in the behavior of virtually adjacent cortical areas.

In these closely spaced cortical areas, DeMott found steep voltage gradients existing at the surface of the brain. A 0.5 mV difference was measured over just 0.5 mm of the cortex which is made up of highly conductive tissue. This result implies that large currents flow in the upper layers of cortex, and shows that electrical activity exists on a very fine spatial scale which can be measured directly from the cortical surface.

DeMott used a device called a toposcope in his study. The toposcope consisted of 400 R-C coupled amplifiers (one for each electrode). The amplifiers were connected in such a way that any simultaneous activity over the entire electrode array would be

eliminated. Only the differences between electrodes in the array would be amplified and recorded.

The output of each amplifier was connected to a neon tube such that the brightness of the tube varied proportionately with the current through it from the amplifier. The 400 neon tubes were arranged in a spatial pattern corresponding to the position of the electrodes so that the resulting light patterns reflected the electrical patterns recorded from the cortex. The patterns were then recorded by photographing the neon tube matrix with a high-speed movie camera about 300 times a second. Analysis of the film allowed voltage levels of each electrode to be quantified, producing a contour plot of the signal amplitudes.

DeMott collected data from the visual cortex of cats, raccoons and tupaia (or tree shrew, which is considered to be a primate of lowly organization); from the auditory cortex of squirrel monkeys and raccoons; and from the somatosensory cortex of squirrel monkeys. Visual stimulus consisted of discrete flashes of light from a flashlight bulb. Auditory stimulus consisted of tones or periodic clicks. Finally, tactual stimulus consisted of the application of touch using an aluminum wire 0.030 inches in diameter with a rounded end.

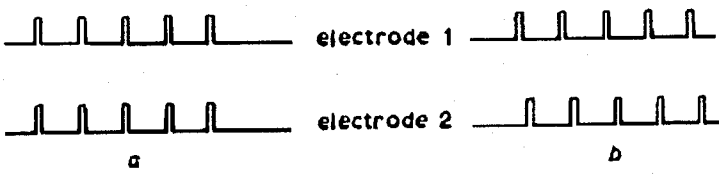
When an appropriate stimulus was presented, DeMott found that cortical activity was displayed as highly detailed patterns in a specific sequence. The peak-to-peak differences usually ranged from 200 to 500 μV. Additionally, if the same stimulus was repeated, the same pattern would repeat as well.

DeMott's data also implied that the entire visual area of cortex participated in the response to visual stimulus (the same type global response was also seen in the auditory cortex in response to a auditory stimulus). He concluded that the visual field was presented to the cortex as a complex spatial-temporal sequence, a theory remarkably similar to that held by researchers today.

A computer–based version of the toposcope was designed by a recent AFIT student [36]. This tool is designed to graphically display signal frequency and phase relationships between different channels of EEG signals. It is possible that the use of this tool may allow additional insight in the analysis of the data collected from the AFIT array.

In 1968 Brindley and Lewin published the description of a multielectrode visual prosthesis implanted in July 1967 on the primary visual cortex of a blind human volunteer [4]. This electrode system used 80 platinum square electrodes 0.8 × 0.8 mm. The electrodes were arranged in a silicon rubber cap which was molded to fit the occipital pole of the right hemisphere. Each electrode was connected to a separate radio receiver coil with adjacent electrodes positioned generally either 3.4 mm (in the middle of the implant) or 2.4 mm apart. The array of radio receivers was encapsulated in a silicon rubber cap designed to be implanted under the scalp. A specific receiver was activated by pressing an external transmitting coil tuned to the receiver's frequency, against the scalp directly above the receiver. The radio signal commonly used in this experiment consisted of 100 pulses/sec each of length 200 μsec. Electrical stimulation of any one of the electrodes produced the subjective experience of "seeing" a point-like light source in a specific location of the subject's visual field. Brindley termed these "phosphenes" and considered them as stimulatable pixels. Thus by stimulating them in appropriate combinations he could transmit crude geometrical images to the visual system of a blind person. Thresholds for phosphene generation, which were estimated to range from 8 V to 56 V, varied depending on the pulse duration and frequency. No estimate of the currents involved for stimulation was given.

Working with Brindley, Donaldson and Eng improved on Brindley's design and performed another implantation in February 1972 [11]. Mechanically, the device was very similar to that used by Brindley except the improved device consisted of two stimulating arrays, one for each hemisphere. The electrical design of the implant



a. Synchronous pulses
b. Asynchronous pulses
Both will cause a pair of phosphenes to be seen for the duration of the train.

Figure 4. Synchronous trains of pulses [11].

was improved by reducing the number of radio receivers needed by the use of a row-and-column matrix. With this design, $m \times n$ electrodes (where m is the number of rows and n is the number of columns) could be controlled by $m + n$ radio channels. It was found that synchronous trains of non-synchronous pulses are as effective as synchronous trains of synchronous pulse in the generation of phosphenes (see Figure 4), hence, stimulus pulses do not have to occur simultaneously for a patient to perceive two phosphenes at once. Furthermore, since the stimulus pulses are brief in comparison to the intervals between them, two or more electrodes could be excited at the same time by scanning the rows (or columns). The 75 electrodes used were made from 50 μm thick pure platinum foil 4×1 mm. Stimulus pulses were 0.2 ms in duration with a period of 10 ms. Unfortunately, the amplitude of the stimulation pulse is not given in the article. In this experiment, the patient was able to read seven Braille letters/minute using six phosphenes.

In 1974, Dobelle and Mladejovsky reported on their investigation of the results of Brindley, Lewin and Donaldson [9]. This investigation involved the use of conscious human volunteers undergoing other occipital lobe surgery. Electrodes were typically platinum disks 1 mm^2 in area on 3 mm centers. Results suggested that changes in the size and configuration of the electrodes or in the stimulus parameters (such as monophasic versus biphasic waveforms, pulse duration, and capacitive cou-

pling), had little effect on the sensation of phosphenes. Also, no difference was noted between monophasic or biphasic waveforms with or without capacitive coupling. All experiments were conducted with constant current circuitry with a typical current of 3 mA being used.

These results were used in further studies by Dobelle and Mladejovsky. Implantation of an electrode array into two blind volunteers was performed in 1974 [8]. The electrode arrays consisted of 64 platinum disk electrodes 1 mm^2 in area arranged in a hexagonal pattern with 3 mm center-to-center spacing. The array was connected to a ribbon cable which passed through the skull. Typical stimulation consisted of symmetrical, biphasic pulses (0.5 msec duration for each phase at 50 Hz) coupled through a 1.0 μF series capacitor. Thresholds for phosphene generation ranged from 0.6 to 2.8 mA (zero-to-peak) in one of the subjects. In a later experiment, the implant was modified by the development of a special percutaneous connector to allow chronic implantation, and by the addition of a CCD TV camera to the stimulation system [10]. In this experiment, a man blind for ten years was able to read Braille at 30 letters a minute, which was faster than he could read tactile Braille. Furthermore the patient was able to detect horizontal and vertical lines with the use of the camera. Again, it was found that there was no differences in phosphene appearance or thresholds based on electrode area. Electrode impedances, however, were found to be complex and nonlinear. For 0.5 msec biphasic pulses, 5.2 – 21.5 V peak to peak was necessary to force 3.0 mA peak to peak current through an electrode.

Semiconductor technology overcomes many of the problems associated with using wire bundle electrodes. Wire electrodes suffer from poor control over their physical and material characteristics, resulting in variation in measurements. Also, the number of electrode sites is limited by the amount of wires which could be passed through the scalp. With the current semiconductor technology, multiplexing of the data is easily performed, greatly reducing the number of connections through the

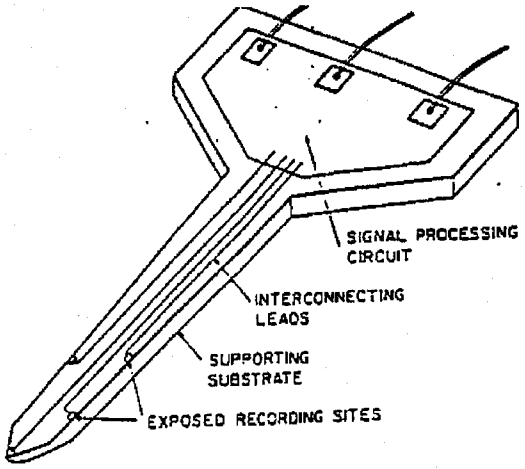


Figure 5. Multielectrode microprobe [3].

scalp. A few of the devices made possible by semiconductor fabrication techniques are discussed in the following paragraphs.

Researchers at the University of Michigan have designed a multichannel multiplexed intracortical probe [3]. This device, shown in Figure 5 resembles a key. The probe is typically 15 μm in thickness with a shank width as narrow as 20 μm. Multiple electrode sites are exposed along the length of the shank. The electrode sites are multiplexed together to allow individual electrodes to be used for recording and or stimulation. By attaching multiple probes in a precisely controlled pattern to an orthogonal platform, a three-dimensional array of electrodes is possible [19]. Such a three-dimensional device can be used to monitor neural activity throughout a volume of cortical tissue. However, use of a intracortical probe does traumatize the tissue. Bleeding was observed during experiments using gerbils. Additionally, during their animal studies, probe electrodes were found to have been coated with biological material (probable protein or fibrin) which may affect the recording capabilities of the electrodes [3].

Researchers at the University of Utah have also developed a three-dimensional intracortical electrode array [23]. This device, shown in Figure 6, consists of a micro machined array of 100 silicon "needles" on a 4.2 $\times$ 4.2 $\times$ 0.12 mm thick substrate.

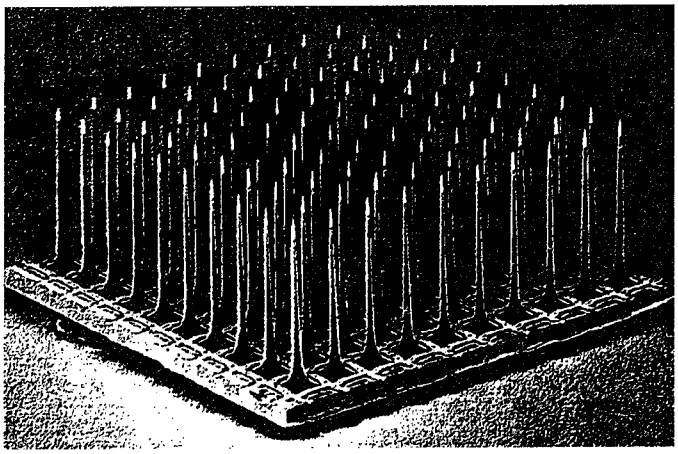


Figure 6. Multielectrode array [23].

The tip of each sharpened needle is coated with platinum and functions as the electrode site. The device is being designed for sensory restoration via intracortical electrical stimulation of a sensory cortex. Experimental implantation of the device has been performed on cats with evidence of some intracortical bleeding due to blood vessel damage. Also, only a few electrodes were able to be accessed with the current technique; a demultiplexer chip to allow access to all electrodes was still in development.

Dagnelie, et al. recorded visually evoked potentials (VEPs) in an alert rhesus monkey using an array of 35 electrodes [6]. The electrodes were organized into five bundles of seven wires each, and were implanted on the visual cortex. The electrodes were constructed by grouping seven Trimel coated 0.06 mm stainless steel wires into a bundle which was twisted and glued together with epoxy. Electrode sites were made by scrapping away 1 mm of insulation from each wire at 6 mm intervals. The wires were attached to a 37 pin connector chronically implanted in the skull. It is worthwhile to note, that in 1989, the date of Dagnelie's article, the array was still in operation five years after implantation. Stimulus consisted of various periodic and aperiodic patterns displayed on a monochrome monitor. The study concentrated on the visually evoked potentials (VEPs) generated by the abrupt onset of checkerboard patterns. The monkey was trained to respond to the presentation of the checkerboard

pattern within 400 ms by pressing a lever. The recorded signals were amplified using a Medelec 50000 system, bandwidth 0.3–75 Hz, fourth–order Butterworth filtered at 70 Hz, and digitized at 240 samples per second. Typically 100 stimulation repetitions were averaged together to produce the averaged VEPs. The VEPs observed usually consisted of a negative peak at 75 ms, followed by a small positive peak at 100 ms and followed by another negative peak at 135 ms. Additionally, although the research was performed as a detailed study of cortical mapping, it also demonstrated the usefulness of surface measurements in the study of cortical processing.

Bartlett and Doty investigated the ability of monkeys to detect microstimulation of the striate cortex [2]. In their experiments, an array of twelve 200 μm Pt–Ir electrodes were implanted 3–8 mm apart within the representation of central vision in the visual cortex of a monkey. The animal was then trained to respond to the application of 0.2 ms electrical pulses at 50 Hz for 2 s durations to its striate cortex. Inter-trial intervals ranged randomly between 5 and 40 seconds. The monkey was rewarded with a sip of fruit juice when he responded by touching a metal rod during the application of electrical stimulus to the striate cortex. The purpose of this study was to measure the threshold for the detection of stimulus. The threshold for detection of the stimulus ranged from 50 to 250 μA, depending on the depth of penetration into the cortex. Bartlett and Doty did not test the animal's response to any visual stimulus pattern to see if the conditioned response could be elicited by a visual stimulus approximating the phosphene pattern which might be expected from the electrical stimulation.

Newsome and Salzman studied the effect of microstimulation (10 μA at 200 Hz via 20 μm intracortical probe), of a directionally sensitive column of neurons in area V5 of a trained rhesus monkey [31]. The monkey was trained on a direction discrimination task using operant conditioning techniques. Their results showed that the application of microstimulation to a column specifically sensitive to a particular direction, significantly influenced the monkey's perceptual judgement of the direction

of movement. A massive increase in the perception of movement in the direction preferred by the stimulated column was noted with microstimulation applied. This result is significant in that it establishes a causal relationship between the activity of direction selective neurons in the cortex and the perceptual decisions made by a monkey on a direction discrimination task. Also noteworthy is the concept that a single, relatively small electrode and stimulus could have such a profound effect on perception.

2.3 Physiology

Much is known about how the brain initially analyzes sensory messages. Yet, how the brain combines sensory messages with past experience for recognition is still not known. Freeman believes that a macroscopic view must be adopted in order to understand perception [15]. His research suggests that perception depends not on the action of individual neurons, but on the simultaneous, cooperative activity of millions of neurons spread throughout the cortex. The behavior of neurons, according to Freeman, can be described by chaos theory, which suggests that there is some hidden order in the seemingly random complex behavior of the brain. Chaos is seen in the response of the brain to the smallest of inputs. Given an input, vast collections of neurons will switch from one complex pattern of activity to another. Freeman believes it is this property of changeability that makes perception possible.

Freeman's studies were concentrated on the neurons of the olfactory system. Action potentials from the receptor neurons in the nasal passages propagate to an area of the cortex known as the olfactory bulb. From there, new signals are sent to many other parts of the brain. Note that this is very similar to the way visual signals are received by the primary visual cortex. EEG data was collected simultaneously from 60 to 64 electrodes 0.5 mm apart attached to the surface of the olfactory bulb of trained rabbits.

EEGs are almost always oscillatory and are usually very irregular. In Freeman's experiments, a "burst" of oscillations could be seen in each EEG tracing when an animal inhaled a familiar scent. All the tracings from the electrode array were suddenly more regular for a few cycles, until the animal exhaled. Also the tracings often had a higher average amplitude (100 μV) and a relatively high frequency (from 20 to 90 Hz). These bursts are labeled as gamma waves (denoting the frequency band of the observed oscillations). Evidence of a collective behavior was shown by the presence of a common waveform, or carrier wave, present in each tracing in the sets of burst recordings. Freeman found that it is not the shape of the carrier wave that identifies an odorant. In fact the wave changed every time the animal inhaled. However, when the average amplitude of each electrode's carrier wave was plotted on a grid representing the olfactory bulb surface, a specific amplitude pattern emerged. As long as the animals' training was not changed the same map resulted for a particular odorant, even though the carrier wave was different for each sniff. If the reinforcement associated with the scent was changed, the amplitude map changed dramatically, which showed the influence of experience on perception. Furthermore, Freeman believes that these amplitude maps demonstrate that perception depends on activity over the entire bulb and that the bulb participates in assigning meaning to stimuli.

Freeman hypothesizes the existence of neuron structures called "nerve cell assemblies". These nerve cell assemblies serve as a repository for past associations and are also crucial for the generation of the collective bulbar burst. In earlier research, Freeman found that when animals are trained to discriminate olfactory stimuli with reinforcement techniques, certain synapses connecting neurons within the bulb and olfactory cortex are strengthened. These strengthened connections, called Hebbian synapses, result in increased output from the affected neuron. During training, a nerve cell assembly, consisting of neurons joined by Hebbian synapses, is thought to form for a particular scent. Thereafter, if the familiar scent is received by any subset

of the neurons in the assembly, the entire assembly can become stimulated, as the excitatory signals are propagated across the favored Hebbian synapses. The assembly then directs the rest of the bulb into the particular pattern of activity. Existence of such structures would help explain how the brain distinguishes one scent from all the others that accompany it (known as the classic Foreground–Background or Figure–Ground problem). In this case, a nerve cell assembly would distinguish a stimulus that has been made important to an individual from experience stored in Hebbian synapses.

Nerve cell assemblies would also help to explain how the brain recognizes that signals from different collections of receptors referred to the same stimulus. This ability is known as generalization-over-equivalent receptors. Due to turbulence in nasal air flow, only a few of the many receptors for a particular odorant are excited during a sniff, and the selection varies unpredictably from one sniff to the next. If nerve cell assemblies exist, they would ensure that information from any subset of receptors would propagate immediately over the entire assembly, which in turn would stimulate the rest of the bulb.

Once a burst in generated, the bulb sends a "consensus statement" simultaneously along parallel axon to the olfactory cortex. Somehow, the olfactory cortex must be able to distinguish the consensus statement from the bulb from the background of other stimuli from the bulb and elsewhere. Freeman believes that the parallel connections between the bulb and olfactory cortex are responsible for making this distinction. Each parallel axon from the bulb branches extensively and transmits signals to many thousands of neurons across the olfactory cortex. Consequently, each target cell in the olfactory cortex receives input from many thousands of bulbar cells. The consensus statement from the bulb can be filtered out since the carrier activity of the consensus statement is synchronized by cooperation, meaning that the synchronized signals will add together. The non-synchronous inputs, which are not at the carrier frequency and phase cancel each other out. The neurons in the

cortex, which have also formed their own nerve cell assemblies, then generate their own collective burst.

Freeman found other parts of the brain may exhibit the same chaotic behavior seen in the olfactory system. In fact, he has documented gamma bursts across large cortical regions involved in the recognition of visual images. His research suggests that familiar visual stimuli are also associated with specific amplitude maps of common carrier waves. Freeman predicts that when viewing a drawing in which foreground and background are ambiguous, such that perception alternates between the two images, the amplitude maps will be found to alternate as well.

Similar oscillatory responses were discovered in the cortex of cats and the macaque monkey [16, 12]. Interburst intervals were found to be between 15 and 35 ms. Engel, et al. found that the responses of the cell assemblies are stimulus dependent [12]. Activity was recorded from four different orientation columns of area 17 of the cat visual cortex. Of the four cell groups, each had overlapping receptive field but different orientation preferences. When stimulated with a single light bar these cells fired synchronously. However, when the stimulus included two superimposed light bars with different orientations and direction of motion, these same cells segregated into two different assemblies each firing in a synchronous pattern that was uncorrelated to one another. Such a result suggests that these synchronous responses allow the distinction of several co-existing representations in the same region of cortex. Hence, how the brain solves the superposition problem concerned with recognizing multiple objects on a complex background may be solved by this "temporal coding" of the oscillatory responses.

2.4 Potential Experiments

From the physiological studies, the well known anatomical data, and the research on human volunteers, it is clear that it is possible to insert subjectively perceivable image-like stimuli into mammalian visual systems. Therefore, it is rea-

sonable to assume that an animal can be trained to respond by, say, pressing a lever to get a food reward, to a simple visual stimuli such as a point of light at a specific location in the visual field. Then the insertion of such a visual form directly into its primary visual cortex by electrical stimulus should result in the trained response. Such an experimental result would verify the integrity of the electronic and physiological systems as well as the experimental hypothesis that direct stimulation of animal visual cortex could duplicate the results obtained in human volunteer subjects.

Success at this stage suggests an even more interesting extension of the procedure. While damage to the human primary visual cortex produces only blind spots in the visual field, damage in secondary visual processing areas of the cortex produces categorical visual deficits lumped under the general term: "agnosias". One of these, as an example, prosopagnosia is the complete inability to recognize human faces, even one's own, while retaining otherwise normal visual perception. When a brain so damaged is recovered after death, the only obvious damage is the destruction of a small piece of cerebral cortex in a secondary visual processing area. This leads to postulating a system where visual data are first mapped to primary visual cortex where no significant form analysis (or "perception") occurs since damage there only causes circumscribed blind spots in the visual field. Following this mapping, the visual data are transferred by cortico-cortical tracts in the cerebral alba to secondary visual areas in the cortex where the elegant analysis we call perception actually occurs. Damage to these secondary regions causes catastrophic perceptual disease. It would be of great interest to monitor primary and secondary visual areas simultaneously in an attempt to gather data while the brain is actually performing perception. This type of study should provide some insight to the nature of the organization of the cortico-cortical connectivity matrix and its relation to the function of the cortical column system [26].

Furthermore, it is reasonable to expect that a monkey trained to respond to a more complex stimulus such as a light bar at a particular orientation could be used to demonstrate that the representation of a stimulus is manifested by a particular gamma burst. If the response to the stimulus was recorded and "played back" through the two dimensional array of electrodes, the monkey should respond as trained just as if the actual visual stimulus was given. Success of such an experiment would depend on the ability to elicit a cortex-wide burst and also serendipitous placement of the electrodes.

2.5 Summary

It is clear that electrical activity exists on a fine scale within the cortex. Furthermore, an electrode resting on the cortical surface is able to stimulate or record the electrical activity of the neurons in close proximity to the electrode. Previous research has been performed using either intracortical probes, which penetrate into the cortical tissue, or surface electrodes, which is the approach taken by the AFIT multielectrode array.

Properties of the intracortical probe concept emphasize the importance of getting more intimate connection to the interior of the cortical column system. They seem to overlook the inevitable damage which these electrodes cause. But the fact that the columns behave as unitary devices leads to an assumption that individual columns can be effectively stimulated from the cortical surface ends of the columns as in fact has been done for decades. Therefore, the AFIT electrode is placed on the cortical surface where it will produce little or no trauma to the cortex beneath it and where it will still have access to the columns for recording and stimulation purposes.

Table 1 summarizes the relevant details from the previous research reviewed in this chapter. From these studies it is reasonable to expect that the AFIT multielectrode array can be used for recording and stimulation of the visual cortex. A recorded signal should have amplitudes in the 100 μV range with a frequency of

approximately 50 Hz. A visual evoked response in the rhesus monkey should have latencies related to that found by Dagnelie, et al. (75 ms, 100 ms, 135 ms peaks) [6]. A stimulus of either monophasic or biphasic constant current, 0.2 ms pulses of 3 mA amplitude at 50 Hz should be sufficient to generate phosphenes in the rhesus monkey. The next chapter discusses all of the relevant steps taken to prepare for a successful completion of the implantation experiment.

Table 1. Summary of Previous Research.

Previous Research			
Researcher(s), Subject(s)	Array Size	Experiment	Comments
DeMott, 1966. cats, raccoons, tupaia, squirrel monkey [7]	400	recorded 0.5 mV difference over 0.5 mm of cortex	demonstrated differences in behavior of virtually adjacent cortical areas
Brindley & Lewin, 1967. humans [4]	80	stimulation, 100 pulses/sec each 200 μs, 8–56V threshold	electrical stimulation produced phosphenes
Donaldson & Eng, 1972. humans [11]	75	stimulation, 0.2 ms pulses with 10 ms period	patient able to read 7 Braille letters/min with 6 phosphenes
Dobelle & Mladejovsky, 1974. human [9]	64	symmetrical biphasic pulses 0.5 ms for each phase at 50 Hz. thresholds 0.6–2.8 mA.	monophasic & biphasic pulses worked. patient read 30 letters/min.
BeMent, et al. , 1986. gerbils [3]	intra–cortical probes	designed for stimulation and recording	lower thresholds for stimulation, but probe harms the tissue
Jones, et al. 1991. cats [23]	100 intra–cortical probes	designed for stimulation and recording	only a few electrodes able to be accessesed by current technique
Dagnelie, et al. , 1989. Rhesus monkey [6]	35	recorded visually evoked potentials (VEPs)	VEPs made-up of negative peak at 75 ms, positive peak at 100 ms then negative at 135 ms.
Bartlett & Doty, 1980. monkeys [2]	12 at various depths	stimulus 0.2 ms pulses at 50 Hz. Thresholds ranged from 50–250 μA	studied ability of monkeys to detect stimulation of striate cortex.
Freeman, 1991. rabbits [15]	60 to 64	recorded from olfactory bulb, bursts of 100 μV amplitude and frequency 20–90 Hz.	recorded gamma bursts of oscillations in response to a familiar scent.
Newsome & Salzman, 1993. Rhesus monkey [31]	intra–cortical probes	microstimulation of direction sensitive columns.10 μA, . 200Hz	massive influence of monkey's judgement of direction of movement.

III. Methodology

3.1 Introduction

The tasks accomplished in this thesis effort can be categorized into several distinct areas. These areas are: encapsulation and packaging, design of the experiment, the protocol, instrumentation, data collection and analysis, and implantation. The following sections of this chapter discuss tasks accomplished and their results for each of these areas. The next chapter presents conclusions and recommendations based on these results.

3.2 Encapsulation and Packaging

To protect the AFIT array from the environment of the brain, several additional processing steps must be performed. These processing steps include coating the aluminum electrodes with a noncorrossive metal as well as passivating the remainder of the chip with several coats of polyimide. The methods used for processing the chips are detailed in Reid's thesis [33] and are outlined in Appendix C.

The implantation package designed by Reid (see Figure 7) consisted of a hollow cylindrical mount which is surgically placed into the subject's skull. The packaged array could be placed inside the cylinder and a protective cap screwed on the top of the cylinder. The intent of this approach was to allow an array which had ceased to function to be replaced without further surgery. However, after consultation with Dr Cooper, DVM, of the Armstrong Laboratory, Comparative medicine Branch, it was determined that such a design would involve a great risk of infection which could lead to encephalitis. Therefore, it was decided that the device would have to be chronically implanted and thus would not be replaceable once surgically installed.

The implantation package designed by Reid was first modified by eliminating the removable inner sleeve. A mini-din type connector was inserted in one end of the package and the mounted array inserted in the opposite end. Although this design

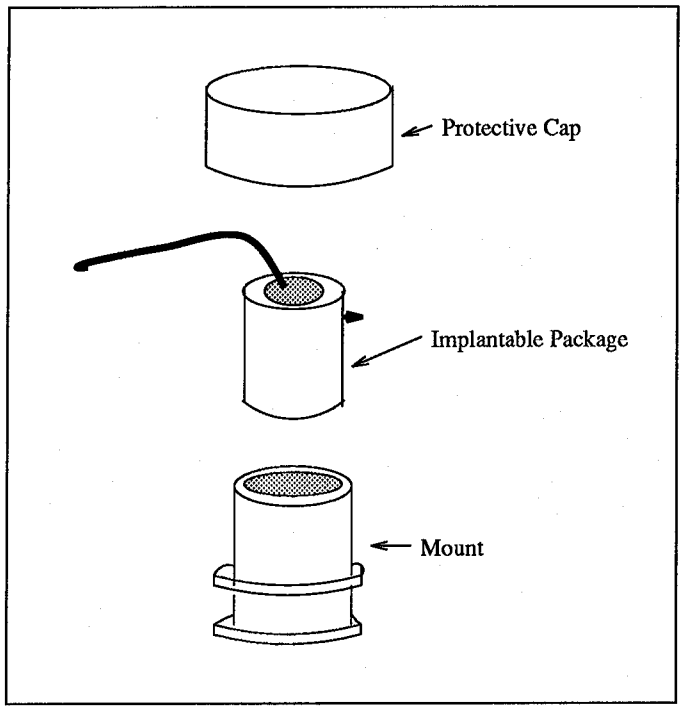


Figure 7. Reid implantation package.

overcame the problem of leaving an opening which could become infected, it suffered from some limitations due to the overall weight of the package and the fact that it could not be used for animals with small skulls or where the curvature of the skull was great. Furthermore, without the feature of a removable inner sleeve, there was no need to have such a large mount protruding from the skull of the experimental subject.

Because of these factors, an approach very similar to that used in the first implantation [26] was chosen. The implantation package chosen is shown in Figure 8. After the three layers of polyimide have been applied to the chip, the device is mounted to a circular header which provides wire bonding posts as well as structural support. The header, shown in Figure 9, is a TO–8 package, 0.6 in in diameter, manufactured by Phillips Technologies, Airpax Protection Group.

Connections are made from the chip bonding pads to the header package pins as depicted in Figure 10. The wire–bonded connections are then covered with two

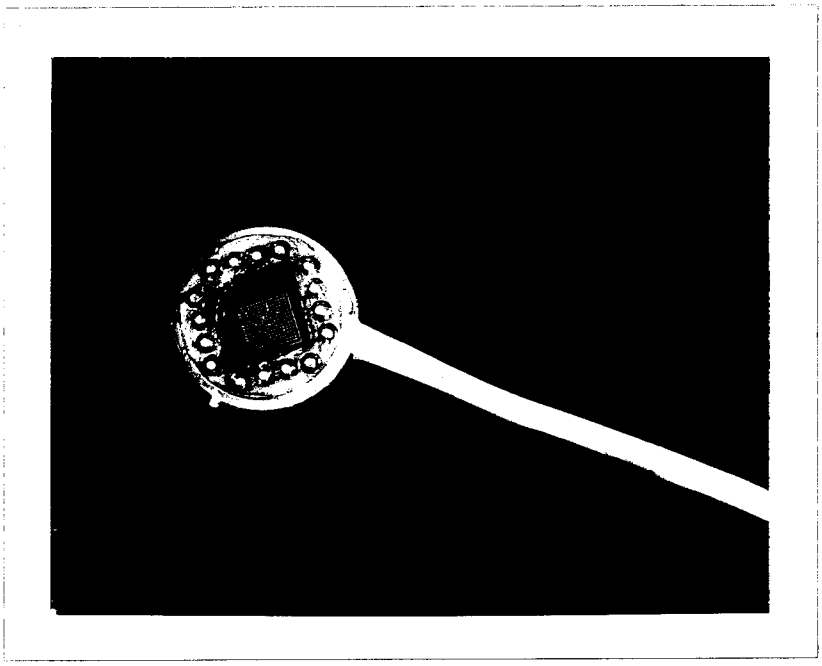


Figure 8. Implantation package.

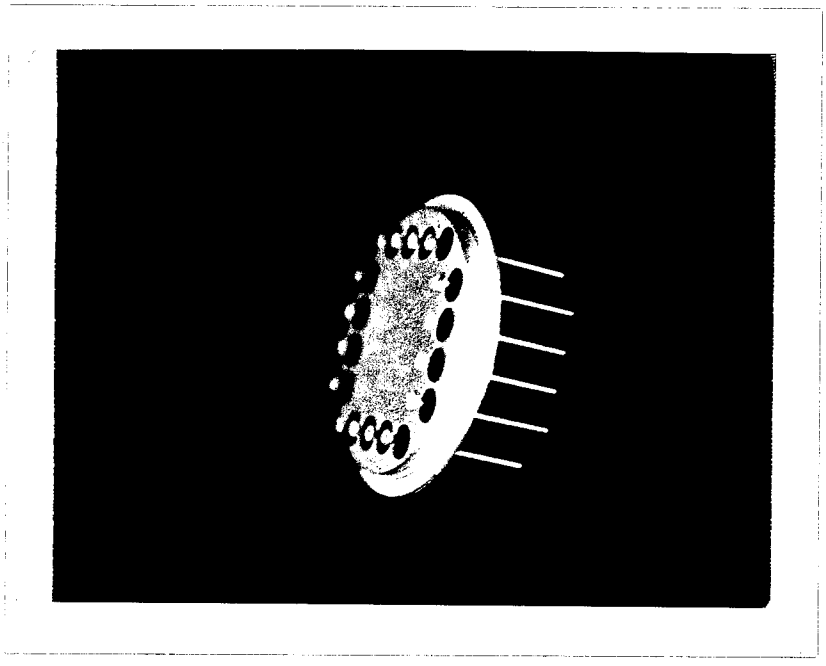


Figure 9. Header package.

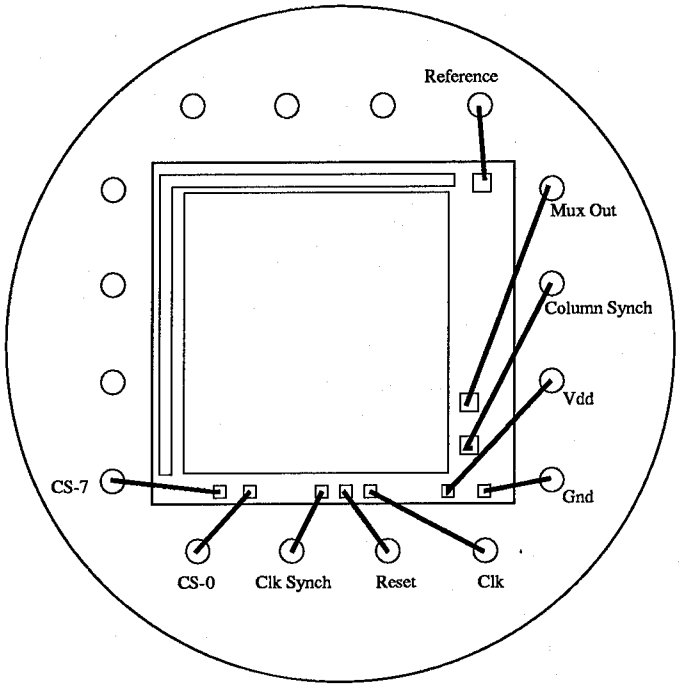


Figure 10. Wire–bonding diagram.

coats of polyimide. A final coating of silicon sealant is applied over the wire–bonded connections to ensure complete coverage and protection.

As can be seen in Figure 10, only 10 of the 16 pins of the header package are connected to the chip. Furthermore, several of the connections (CS-7, which is the eighth bit of the counter, CS-0, which is the first bit of the counter, and CLK synch) are used only for testing of the chip and are not necessary for normal chip operation. Therefore the pins connected to these unnecessary signals and the unused pins are clipped level to the back surface of the header. The remaining pins are clipped to approximately 3 mm in length and are each soldered to different colored solid–core 30–gauge wire. Connections are made easier by using a wire–wrap tool to wrap the wire around the header pins prior to soldering. Table 2 lists the colors used for each connection. Next, a thin layer of silicon sealant is applied to the soldered connections and allowed to dry. This application of silicon sealant is to ensure that the connections will be insulated from one another as well from the body of the

Table 2. Color Coding of Wire Connections.

Connection	*Wire Color*
reference	deep yellow
mux out	pale yellow
Vdd	red
GND	black
clock	blue
reset	green
col synch	white

header itself. The pins are then carefully bent over towards the middle of the header to reduce the overall width of the implantation package. The wires are then grouped into a bundle using heat shrink tubing. A final coat of silicon sealant is applied to the back of the header to further insulate and secure the wire connections. Cable lengths for the ferret and rhesus monkey are approximately 8 and 12 inches respectively

The packaged device is connected to an external 8–pin female mini–din type connector. However, since the cable must be routed under the skin, the connector cannot be permanently attached until after implantation. The connection scheme of the connector is shown in Figure 11.

3.2.1 Discussion. Post-processing of the chip was an extremely time–consuming task. Since the devices are received from MOSIS as individual chips, they must be processed one at a time through all of the post–processing steps. These post-processing steps were performed at the AFIT Cooperative Electronics, Materials, and Processes Laboratory. The experience has provided several important lessons learned that will improve the yield of functional implantable devices. These lessons are discussed in the following paragraphs.

Some of the materials used were past the manufacturers recommended expiration date. This may have caused some problems with the quality of both the positive and negative photoresist. This did not prove to be too much of a concern if one was

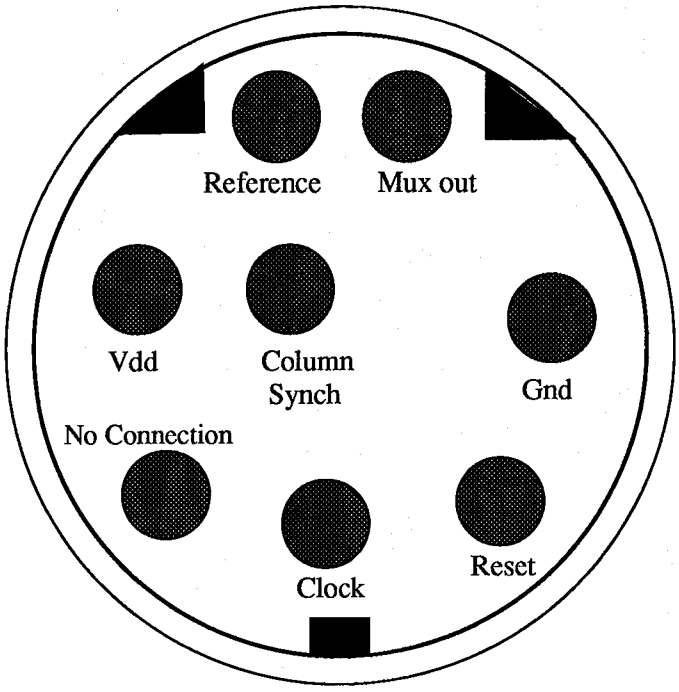


Figure 11. Connection scheme for female connector.

careful to check the condition of the chip through a microscope after each step. If a problem with the material is observed, in most cases it can be removed and redone.

Best results were achieved when the polyimide, which was stored in a refrigerator, was allowed to reach room temperature prior to use. The polyimide layers tend to remain slightly sticky until the final cure. It was discovered that if strong contact is made with the mask of the mask aligner, the polyimide layer may stick to the mask and be peeled off at the edges of the chip. If this occurs, the polyimide must be removed using DuPont T–9039 thinner and light pressure with a cotton swab. Therefore, the mask aligner should be set such that the mask is only in light contact with the chip.

Mask alignment was made much easier by placing the chip on the sticky part of a 3 inch post–it style note. The adhesive on the paper helps to keep the chip from moving or sticking to the mask. The paper, which extends out from under the alignment stage can also be used to properly position the chip quickly for alignment.

After the chip is mounted on the header package, the wire bonds are protected by the application of polyimide over the wire–bonded connections. Since, this application is made with a toothpick, extreme care must be taken to prevent damage to the device. Obviously, the toothpick could cause wire–bonds to be broken if care is not used. Additionally, the devices can be ruined by the application of too much polyimide. Generally, the polyimide tends to have a relatively high meniscus and does not spread across the surface of the chip. However, this may not be the case, if too much is applied, the polyimide may spread over electrode surfaces as it is being cured. At this stage of processing the device is essentially ruined and cannot be used.

The implantation package is intended to be as small as possible. This requires the use of very small–gauge wire for connection to the chip. Working with the solid-core 30–gauge wire which was selected, posed several difficulties. If the wire was nicked when the insulation is being stripped, it had a tendency to break at that nick. The use of shrink tubing over the stripped portion of wire helped to prevent wire breakage.

3.3 Design of Experiment

3.3.1 Potential Experiments. The design of the AFIT array has gone through many changes since the first implantation [1, 39, 37, 28, 33]. These changes include a decrease in the size of each electrode as well as an increase in the number of electrodes from 16 to 256. Therefore it is very important as a first step to verify that these changes to the structure of the electrodes will still allow EEG measurements to be made. This is the first experiment that will be performed. The packaged device will first be implanted on the primary visual cortex of a test subject and the signals recorded by the device will be demultiplexed and evaluated. It is expected that EEG tracings will be observed.

To further demonstrate the operation of the array, an attempt to record visual evoked response (VER) to a flashing light will be made. The stimulus will be produced

by a xenon stroboscopic lamp. Empirical evidence supports the use of stimulus frequencies of three or less flashes per second [24]. At this frequency the response trails of each evoked response should not overlap sufficiently to add constructively. The VER measurement will be made by averaging 100 EEG signals, each recorded immediately following a flash. By averaging the EEG signals the VER is extracted from the normal background cortical activity. This extraction method assumes that there is a consistent response to the stimulus which is synchronized with the strobe. Since the background cortical activity could be considered to be random in relation to the strobe, summation of EEG signals should result in the VER constructively adding while the background activity should not add constructively. In humans, a VER will show a peak of activity occurring in the visual cortex approximately 100 ms after the image is presented; a second peak occurs at approximately 300 ms [32]. A similar response should be seen in other mammals with the difference being the latencies between peaks.

Success of this first phase of experimentation would be followed by implantation of the array into a primate. Logically, the recording of EEG and VER data would be repeated for the primate in order to verify the operational status of the implanted array. The remainder of the experiments to be performed would depend on the success at obtaining trained responses from the monkey and the duration of time that the device continues to operate. At the very least, an attempt to record a gamma burst type response to a moving light bar will be made. If the primate is trained to associate a light bar moving in a particular orientation with a reward such as a sip of juice, it should be possible to find a gamma burst type response. The signal recorded by each separate electrode would be examined for the presence of a common waveform with a higher amplitude and frequency (20 to 90 Hz). If present, then a plot of the average amplitude of each electrode's carrier wave should yield an amplitude pattern similar to those found by Freeman in his study of the olfactory system of rabbits [15], see Figure 12. The amplitude pattern should remain the same

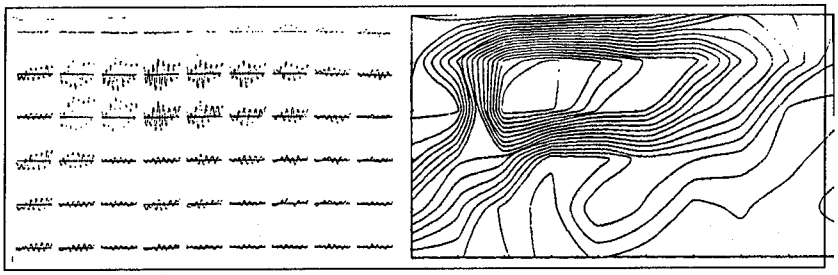


Figure 12. Gamma burst recordings and the resulting amplitude plot [15].

for successive trials as long as the reinforcement for recognition of the pattern is not changed.

3.3.2 Evaluation of Potential Experimental Subjects. As mentioned earlier, the first implantation was performed with a laboratory beagle as the experimental subject [26]. While excellent data were recorded from this animal, certain considerations make the use of a dog as an experimental subject undesirable. Most importantly, although the experiments performed by AFIT are benign to the animal, use of a dog or even a cat in any experiment might cause a large amount of public disapproval. Therefore, it was necessary to evaluate the different types of animals available for experimental research to determine which would best suit the proposed study.

With the ultimate goal of better understanding the process of human visual recognition, the use of a non-human primate as the optimal experimental animal seems obvious. Use of a primate for this study would have four key advantages. First, the study requires a species with a brain in which the visual cortex is surgically accessible and of sufficient size to allow placement of a 0.5×0.5 cm chip. This requirement was shown to be satisfied in rhesus monkeys by the performance of a 'dry run' of the planned surgery by Dr Cooper, DVM, on a rhesus monkey which had died of natural causes. Second, later stages of this research will require a species which can be readily trained to respond to visual images originating either through the normal optic pathways or via electrical impulses delivered directly to the visual

cortex. As described in the literature review, other researchers have had great success in training rhesus monkeys to respond to such stimuli. Third, use of the non–human primate allows more efficient use of data already collected in humans. Conversely, the fourth advantage is that data from this study should be directly applicable to the human species.

However, before any experimentation is performed on a primate, the chip's operation must be tested. Therefore, it was decided to verify the correct operation of the chip using the lowest phylogenetic species of animal feasible. The animals evaluated were those that are commonly used as laboratory research subjects. The rat, which is a very common laboratory animal did not have a large enough skull or brain to accommodate the array. A necropsy of a pig revealed an extremely thick skull over the visual cortical area. The skull measured approximately 2 cm thick and it was determined that there would be too much risk of extensive trauma involved to implant the device.

Review of experimental animal research literature revealed that the ferret has been shown to be very useful in the study of visual systems [21]. Researchers have found that the ferret closely resembles a dog and has features that make it suitable as a useful research animal [40]. A lateral view of the ferret skull and a simplified lateral view of the brain are shown in Figure 13. The approximate location of the primary visual cortex is shown by the stippling in this figure. It is believed that a device implanted through an opening near the rear of the skull should rest in part on a section of the primary visual cortex. The ferret was also found to be easily acquired and was therefore selected as the experimental subject for the first phase of the proposed study.

3.4 Protocol

As previously discussed, approval of the protocol was essential to the performance of this study. Local guidance concerning the protocol stated that the approval

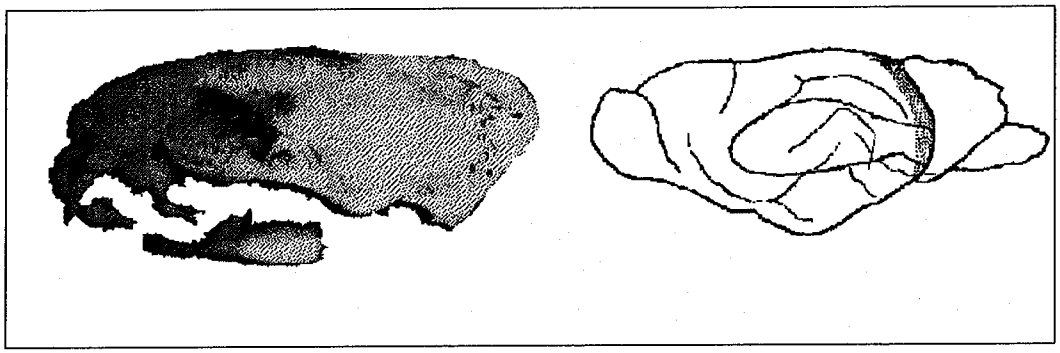


Figure 13. Lateral views of the skull and brain of the ferret [21, 40].

process should only take about 30 days. However, this was proven not to be the case. The protocol was originally submitted to the Armstrong Laboratory Animal Care and Use Committee (ACUC) the first part of August, 1994. The ACUC conditionally approved the protocol 17 August, pending several modifications. Once these modifications were made, the revised protocol was resubmitted on 31 August. This revised protocol was then approved by the ACUC and forwarded to the Air Force Non–human Primate Committee at Brooks AFB on 16 September. The committee approved the protocol pending minor modifications which were made prior to submittal of the protocol to the Office of the Air Force Surgeon General on 30 September. Final approval of the protocol was made by the Office of the Air Force Surgeon General on 3 October and was received at AFIT 12 October.

3.5 Instrumentation

3.5.1 Recording. As mentioned earlier the goal of the design for instrumentation setup was for it to allow quick and easy use of the data. The key to accomplishing this goal is the use of a LeCroy 7200 Precision Digital Oscilloscope to record the measured signals. This oscilloscope provides high accuracy waveform measurements and analysis with built–in digital storage memory [27]. The displayed traces can be recorded in an internal four Mbyte (two million data points) non–volatile circular buffer. These recorded traces may then be replayed, examined, processed, or stored to an MS–DOS compatible floppy disk. The oscilloscope is also

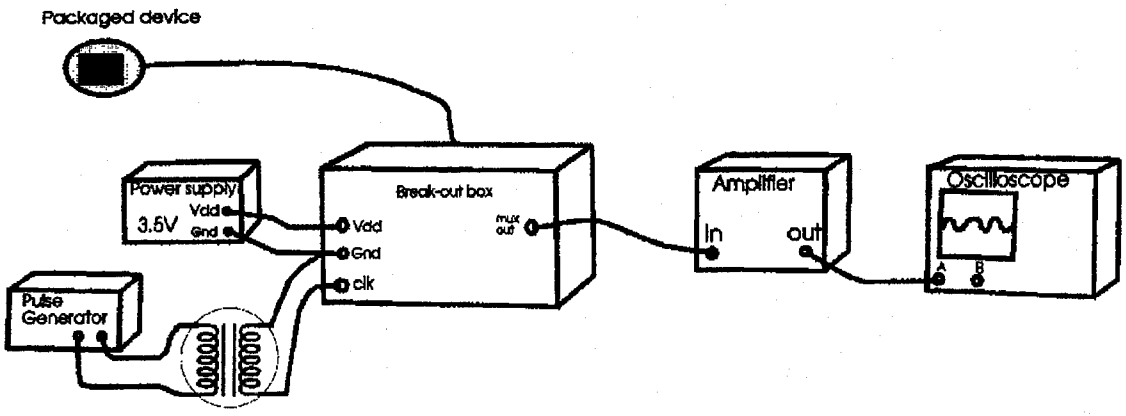


Figure 14. Block diagram of instrumentation setup.

capable of being operated through a GPIB or RS232 bus which allows remote operation and processing of data. Alternatively, a tabular printout of the waveform data points and acquisition information can easily be produced. The specific settings of the oscilloscope controls will be presented in a later section. The following paragraphs discuss the rest of the instrumentation setup used for recording cortical signals. Figure 14 is a block diagram of this setup.

There are five necessary connections to the chip. These connections are: Vdd, ground, reset, clock, mux out, and reference electrode. Power at 3.5 V is provided by a Powertec 6C3000 DC voltage power supply. To reduce noise a battery operated power supply has been designed which can be substituted for the Powertec power supply. The schematic of the battery operated power supply is shown in Figure 15.

The clock is provided by a Hewlett–Packard (HP) 8082A pulse generator set with a 50% duty cycle and a peak to peak voltage of 2.0 V. Since typical EEG signals are below 50 Hz, each electrode must be sampled at a rate equal to or greater than the Nyquist rate of 100 samples per second. Since there are 256 electrodes and the counter increments at half of the external clock rate, the clock must be set at a minimum of 51.2 kHz to satisfy the Nyquist rate criteria.

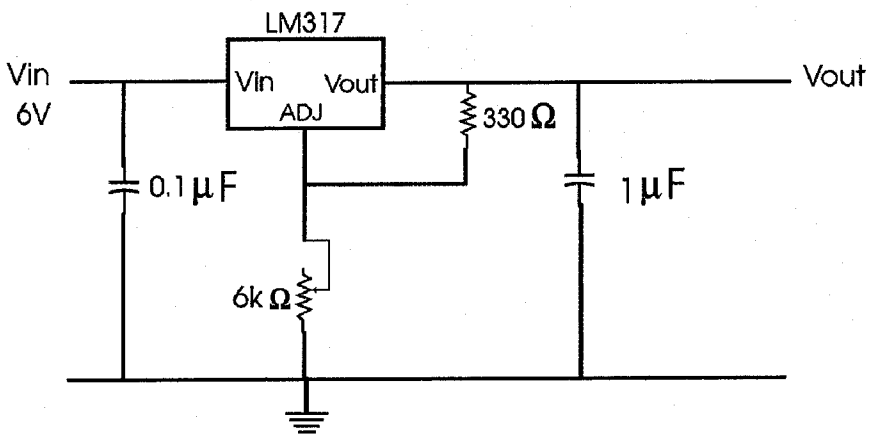


Figure 15. Schematic of battery operated DC power supply.

The reset line could be connected to either ground or Vdd through an external break–out box. The break–out box, pictured in Figure 16, provides a method to connect to the packaged chip through a male 8–pin mini-din style connector.

The multiplexed output and reference electrode are connected to the input of an amplifier. The amplifier used is a Stanford Research Systems low–noise preamplifier, model SR560. The amplifier is set to bandpass 0.03–30 kHz, with a dropoff of 6 dB/oct. The bandpass filter must be set this high because, although the signals of interest are in the 50 Hz range, the output of the chip is multiplexed at one half the frequency of the external clock, approximately 25 kHz. The adjustable gain can be set from 1 to 5 $\times 10^4$.

During recording it is important to monitor the output of the amplifier to ensure that amplifier *blocking* does not occur. Amplifier blocking refers to the saturation of or overloading of the amplifier for a period of time due to the passage of a transient signal with a relatively high magnitude. This condition is signaled by the illumination of a red 'overload' light on the amplifier and may require a reduction in the amplifier gain in order to unblock the amplifier.

Recording the VER requires precise timing and synchronization. For VER measurements, a xenon stroboscopic lamp was purchased from Radio Shack. This

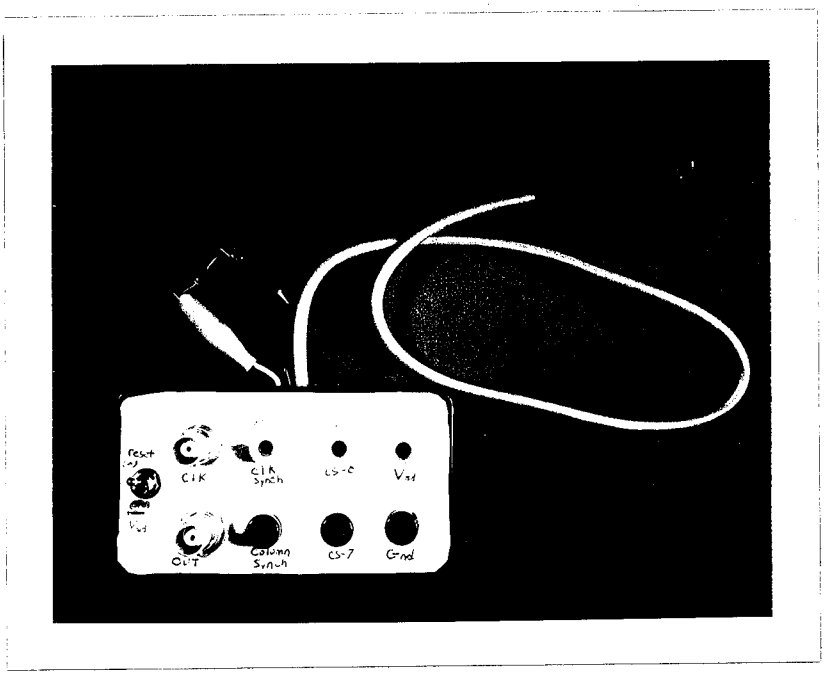


Figure 16. Break–out box.

lamp produces approximately 10 to 40 μsec pulses of intense light which can be presented at variable rates. Changes in the intensity of the flashes will affect the VER. Increasing the intensity of the light stimulus produces VERs characterized by greater complexity and amplitude and shorter latencies [24]. Since the stroboscopic light purchased is not considered to be a precision instrument, it is possible that the energy output of the xenon light may vary from flash to flash. However, since the VER is an *average* response to a number of individual flashes, this flash–to–flash variability (if present) should not be a problem. If the total number of flashes averaged to produce a VER is considered to be great enough to encompass the entire range of flash variability, then the variability should contribute minimally to the differences between similarly produced VERs.

For a VER measurement, the exact timing of each strobe pulse in relation to the recorded cortical response is necessary. To determine the timing of the strobe flash, a photodetector device was designed, constructed and found to function as designed. The photodetector device outputs a pulse for each flash from the stroboscopic lamp.

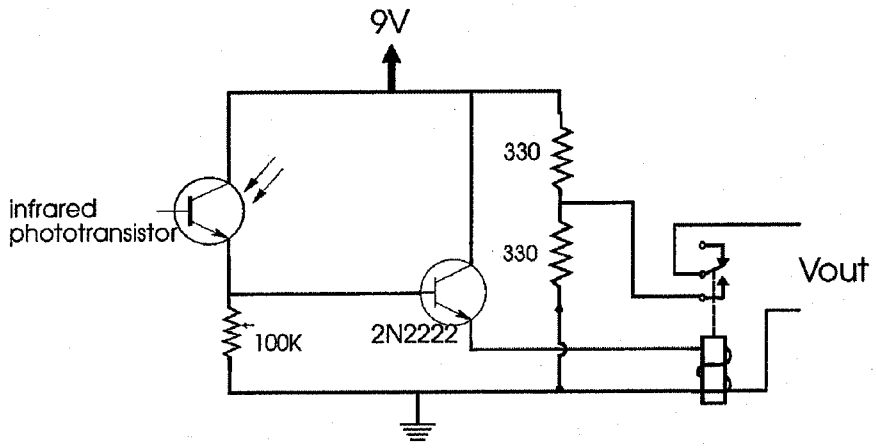


Figure 17. Schematic diagram of photodetector circuit.

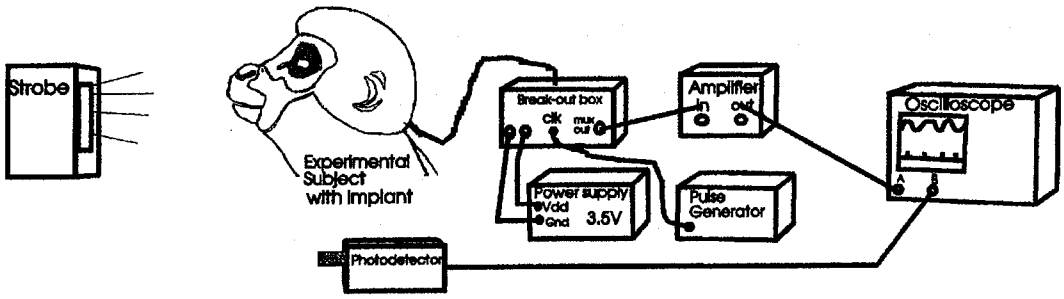


Figure 18. Block diagram of VER data collection setup.

The schematic for the photodetector is shown in Figure 17. In order to obtain a VER, the amplified output of the chip and the output of the photodetector device are recorded for approximately 100 flashes (as shown in Figure 18). The data is then demultiplexed to separate the signals into individual array electrode measurements. The output of the photodetector is used to determine the timing of the strobe flashes. The evoked activity between stimuli is then averaged for each electrode in the array. The resulting average of the nonrandom activity is the VER.

To ensure that the recorded response is a VER, a session should be conducted in which the flashes from the strobe light are obscured from the experimental subject. This trial will be used as a control to make sure that the strobe light is not induc-

ing non-biological artifacts into the recorded VER. A VER obtained in the manner described should yield a good estimate of the evoked response. The measurement is considered to be only an estimate recognizing that the waveform obtained results are from the average evoked response plus the average ongoing bioelectric background activity as well as added interference or artifacts [24].

3.5.2 Stimulation. For stimulation it was determined that a constant current source would be necessary, which is most easily approximated for short periods of time (pulses). Additionally, evaluation of the design of the AFIT multielectrode array revealed that the electrodes may not be capable of providing stimulus signals with negative potentials. Each electrode is connected to the output through two CMOS transmission gates. A transmission gate allows bidirectional flow of signals when on and no flow when off. It operates essentially as a voltage controlled switch. Operation of a CMOS transmission gate requires the input and output voltages to fall within a certain range, generally between the most negative terminal *Vss* and the most positive *Vdd*. Signals outside of this range could cause the gate to prematurely turn off or damage the gate. In the AFIT array, the device ground is connected as the *Vss* input for the gate. The consequences of this design are difficult to predict due to the small signal (relative to *Vdd*) being passed and also due to the fact that the ground references are not common for the transmission gate control voltages and the signal passing through the gate. Thus, it is possible that inserting a negative pulse through an electrode may cause damage to the device. Therefore, only a positive monophasic pulse will be used as a stimulation source with the AFIT array. From the literature review it was clear that a monophasic pulse could be used to stimulate the cortex, therefore this restriction still allows the AFIT array to be used in stimulation experiments. The parameters which should provide useful stimulus consist of 0.2 ms pulses of 3 mA amplitude at 50 Hz.

3.5.3 Testing of Instrumentation Setup. Previous testing of the AFIT array showed that the device could be used to record signal with 3–5 mV amplitudes [35]. However, from the review of the literature, it was determined that an ability to record signals with amplitudes as low as 20–500 μV was desired. Therefore, further testing of the AFIT array was necessary to determine the capability of the device.

The first step was to determine whether the instrumentation setup was able to display and record the desired signal levels. To conduct the testing, a simulated brain environment was constructed using a paper towel saturated with a 0.9% saline solution (which is similar to the saline content of the cerebral spinal fluid). A sinusoidal signal was applied across the paper towel. Signal amplitudes could be adjusted such that a very low level signal was available for input from the surface of the paper towel. A test jig with two electrodes approximately 0.5 mm apart was used to simulate a single electrode and the reference electrode of the AFIT array. The electrodes were placed on the paper towel and connected to the amplifier. Both single–ended mode and differential mode connections to the amplifier were tested. It was determined that differential mode was the most effective in isolating a signal from the noise present. This was to be expected since with differential mode amplification the noise common to both electrodes is rejected and only the activity which differs at the two leads is amplified.

In the differential mode, recording of a very clean signal in the 10 μV range was accomplished. The signal was amplified with a gain of 5 $\times 10^3$ and bandpassed at 0.03 – 30 Hz. In single – ended mode operation, signals with amplitudes in the 20 μV were easily recognized but had a slightly 'noisy' appearance.

As mentioned earlier, the bandpass filter of the preamplifier must be adjusted to allow the multiplexed frequencies through. However, the AFIT array does have a *reset* capability which connects the output of the device to a single electrode. Therefore, enabling the reset and setting the bandpass filter to the values used in the previous paragraph, should allow the cortical activity from a single electrode to

be observed in real time. The next logical testing step was to test the actual device to see if similar results could be achieved.

Prior to final packaging of the device, the counter circuit of each chip was tested to ensure that this section was operating correctly. A test board capable of interfacing to all wire–bonded connections of a header mounted device was constructed and used to verify that an external clock signal would correctly drive the CS–0 and CS–7 column outputs of the counter. Correct output should represent a high degree of confidence that the counter and decoder circuits are functioning. Chips that failed this initial test were not processed any further. Chips that did pass were then processed through the remaining encapsulation and packaging steps as outlined in Appendix C.

After packaging, the devices were further tested using the break-out box described previously. Each device was tested by putting them in the simulated cortex. Since this test involved immersing the device in a saline solution, it was very important to thoroughly rinse the devices with de-ionized water after testing.

The devices were first tested to ensure that they were capable of recording signals from a single electrode. This is accomplished by setting the reset switch on the break–out box to ground, which causes only the output of electrode (1,1) to be connected to the mux out line. A 900 mV signal was measured at the preamplifier output with a gain of 5 $\times 10^4$, bandpassed at 3 – 30 Hz. This equates to an input signal of 18 μV which is similar to the levels recorded with the test jig. The signal did have more noise present than that of the test jig measurement.

In order to reduce noise, differential–mode amplification of the measured signal from the AFIT array was attempted. The multiplexed output of the device was connected to channel A of the preamplifier and the the reference electrode was connected to channel B. Unfortunately, the impedance of these two electrodes were significantly different. This resulted in very little common noise being rejected by the preamplifier.

By adjusting the bandpass filter to an upper limit of 100 kHz, it was discovered that some of the noise was resulting from the external clock signal which was found to be present (at low amplitudes) on the multiplexed output line. The clock pulses were slightly rounded in appearance indicating passage through some sort of RC circuit. The rise time was measured to be 0.5 μs and since there was a 2.2 kΩ resistor placed between the mux out and reference line, the equivalent internal chip capacitance was estimated to be 23 pF.

When the reset switch is set to *Vdd* the device electrodes are switched at one half the frequency of the external clock. Adjusting the bandpass filter of the preamplifier to accommodate this frequency also requires that the gain be decreased to prevent an overloaded condition on the amplifier. In this mode, the presence of the input signal in the output of the device is not obvious from observation of the oscilloscope trace. Analysis of these multiplexed signals require demultiplexing of the recorded trace.

3.5.4 Instrumentation Settings. Table 3 lists the adjustments which should be made to each equipment item for the conduct of the experiment.

3.5.5 Discussion. The design of the instrumentation was satisfactory. This was proven by the ability to record a 18 μV signal from the multielectrode array. Some difficulties were encountered in the testing of the instrumentation and devices. Determining what the ground loops were proved to be a difficult but necessary experience to ensure isolation of the array. Also, it was very important to have a very methodical approach to get the test setup working correctly. This was accomplished by a step–by–step approach in which only one variable was changed at a time. In this fashion it was possible to isolate various sources of problems such as broken connection wires or intermittent blocking of signals through the isolation transformer in the simulated cortex. Without such an approach it would have been very easy to make wrong assumptions about the setup and the AFIT array.

Table 3. Instrumentation Settings.

Equipment Item	*Adjustment*
DC power supply	Adjust output to 3.5 V
HP 8082A pulse generator	1. Adjust pulse to square wave output 2. Adjust pulse amplitude to 2.5 V 3. Adjust frequency to 52 kHz
SRS SR560 preamplifier	1. Set input channel to channel being used 2. Set mode to 'High Dynamic Range' 3. Adjust bandpass to 0.03 – 30 kHz 4. Adjust gain to the highest level possible without overloading. Be sure to record this value with the recorded trace.
LeCroy 7200A oscilloscope	1. Clear memories a.) Press 'Configure System' softkey b.) Press 'Clear memories' softkey c.) Press 'Yes, Clear memories' 2. Set acquisition parameters a.) Press 'Status' button on the 7242B plug-in b.) Press 'Status' softkey c.) Set Channel A as follows: BW limit: ON Enhanced resolution: 8 bits Max memory: 1M × 2 channels Press 'Return' softkey d.) If performing VER measurement, repeat step c. for channel B. 3. Set timebase to 1 s/div (sample rate of 40 K samples/sec) 4. Press 'return' softkey.

3.6 Data Collection and Analysis

3.6.1 Demultiplexing. Demultiplexing of the recorded signal is performed by computer processing. The recorded traces from the LeCroy oscilloscope are converted into an ASCII table of values by a PC compatible utility program, 'wavetran.exe', which was supplied with the oscilloscope. The output of this program is a single column of numbers representing the amplitudes of each sample. The program can also be used to identify the acquisition data associated with a given trace such as sample rate and the total number of samples recorded.

A simple MATLAB^{TM} (Math Works. Inc.,South Natick,MA.) script was written to extract the signal samples for a particular electrode in the array. The script is written such that each point is assigned a time value which is used to determine what electrode to assign the sample to. The script is presented as Figure 19. This script file produces an ASCII file with two columns of values. The first column contains the time at which the sample occurred (relative to the start of the trace) and the second column contains the amplitude of the signal in volts. After extracting the data for a single electrode, the output file generated can be easily plotted.

3.6.2 Signal Averaging for VER. In order to improve the signal to noise ratio of the VER, 100 signal samples are averaged together. This process is performed by computer post–processing of the collected data. In a VER experiment, the output of the photodetector will be recorded along with the output of the chip. This photodetector output can then be used to provide a time reference to identify the individual segments for averaging.

3.6.3 Discussion. In order to test the demultiplexing procedures, signals were recorded and processed from the simulated cortex. It was noted that the actual number of points recorded by the LeCroy oscilloscope is not necessarily equal to the maximum number of points selected in the oscilloscope setup. For example, although the maximum number of points was set to 500,000, a recorded 10 Hz signal,

```
% A M-file to extract the samples for a given electrode
file = input('Enter name of data file in single quotes: ')
f = input('Enter external clock frequency: ')
h = input('Enter sample period in seconds: ')
e = input('Enter desired electrode number (1 - 256): ')
o = input('Enter name of output file in single quotes: ')
name = file
eval(['load ',name,'.dat'])
k = max(size(eval(name)))   % # of data pts
i = 0:(k-1);          % range variable to # of data pts
s = 1/(f/2);          % electrode switching rate
ep = s/h ;            % # pts per electrode
a = s * 256 ;         % array sample time
n = round((k*h)/a); % # of frames
p = a/h ;             % # of pts per frame
N = 1:n;              % range variable # of frames
T = (0:h:((k*h)-h))'; % generates a column vector of time refs
data = [T (eval(name))]; % combines data with time ref
j = 0                  % initialize loop
c = 0                  % initialize inner loop
while (j < n)
 ltop = round((e-1)*ep+(j*p));        % 1st data pt this frame
 lbottom = round(((e+1)*ep+(j*p)+1)); % last data pt this frame
  if (ltop < k)
    if (lbottom <k)
for c = ltop:lbottom;
  if(((data(c+1,1))>(((e-1)*ep*h)+(j*a)))*
         ((data(c+1,1))<(((e+1)*ep*h)+(j*a))))
     O = [O;data(c,:)];
      c =c + 1;
        else
      c = c + 1;
  end
end
     end
   end
 j = j + 1;
end
eval(['save ',o,'.dat',' O /ascii'])
plot(O(:,1),O(:,2))
```

Figure 19. MATLAB^{TM} script file for demultiplexing.

10 seconds in duration, consisted of only 400,000 points. According to the LeCroy operation manual, the actual number of points will depend on the panel settings such as timebase, horizontal expansion, and processing function [27]. In addition, the binary file for this recorded trace was 800 kbytes. To record the longest possible duration of a trace, the maximum number of points, which is one million points per trace, will be selected. However, since this file will be over twice the size of the file containing 400,000 points (1.6 Mbytes), it can not be transferred from the oscilloscope by use of floppy disks (which only have a 1.44 Mbyte capacity). Therefore, during the experimental sessions, either the recorded traces can not be over 500,000 points or the oscilloscope will have to be connected to a microcomputer through its existing GPIB or RS232 port.

When recorded traces were first processed, the resulting output traces appeared as if the oscilloscope was unable to trigger on or record the input signal. In each trace processed, the trace would appear as expected up to a certain point where the output became erratic. Initially, the cause of this behavior was not known. Therefore, multiple traces with slightly different connections and oscilloscope adjustments were individually recorded and processed to help isolate the cause of the problem. The different traces included measurements with the AFIT array as well as the test jig mentioned previously. It wasn't until these traces were compared to each other that the remarkable similarity between traces was noticed. This result, along with the discovery that the erratic section always began at the 50,000th point, led to the identification of the binary – to – ASCII conversion as the source of the problem. The problem was solved by using a more recent version of the 'wavetran.exe' program from the LeCroy Corporation, as shown in Figure 20. The top graph in this figure is the demultiplexed signals for electrode number one, recorded from the AFIT array with an input signal of approximately 15 mV peak-to-peak. The bottom graph in the figure shows this same trace between the range of 0 to 1 second. Note that the 10 Hz input signal is easily recognized. The amount of noise in this trace seems to be

caused by 60 Hz line noise. When this trace was recorded, the amplifier was being operated on line power rather than its' internal batteries.

3.7 Implantation

The surgical procedure will be to open a small circular opening in the skull over the visual cortex. The dura membrane covering the brain will be opened and the packaged device will fit into this opening. The array will then be resting on the pia membrane. The entire package will then be immobilized using a polymalient glass isomer cement, which will also fill in the gaps between the packaged device and the skull. Three stainless steel bone screws, used to help anchor the implantation package will be tapered into the skull at approximately equal distances around the circumference of the opening. Liquid dental acrylic will then be poured over the exposed surface of the skull and molded so as to provide a solid anchor. Once the dental acrylic has hardened, the skin and subcutaneous tissues will be closed using sutures. The interface cable will be threaded under the animal's skin and brought out from beneath the skin at the shoulders. The cable will be attached to an eight pin mini-din style female connector which will be secured to the animal by a custom fit nylon vest. The animal will be treated by antibiotics prophylactically for the duration of the implantation to prevent wound infection.

Due to time constraints, the implantation of the AFIT multielectrode array could not be accomplished during this thesis effort. However, at the completion of this thesis effort, all preliminary preparations for the performance of the experiment have been made and implantation is only awaiting fabrication of additional chips.

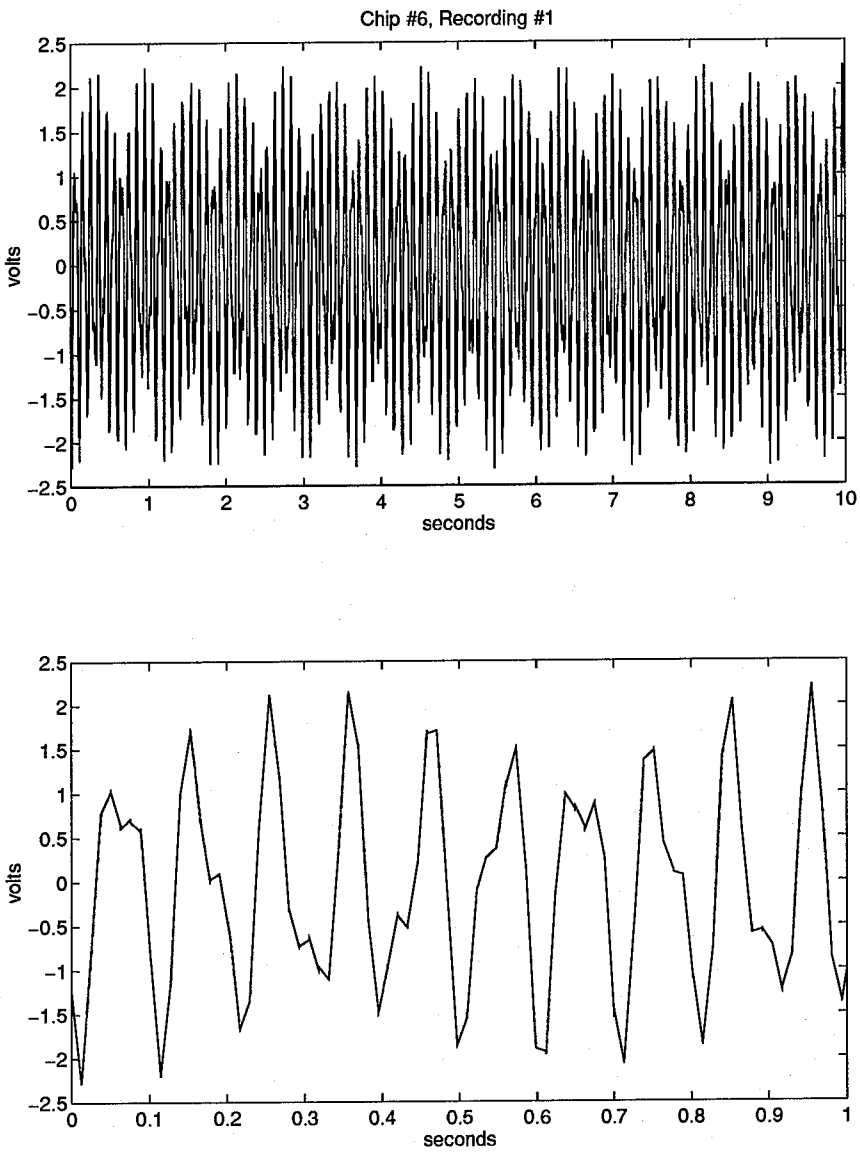


Figure 20. Correct output of demultiplexed trace.

IV. Conclusions and Recommendations

4.1 Current Status

At the beginning of this thesis effort, many details related to the implantation of the AFIT multielectrode array still had not been addressed. Each of these details was an impediment preventing the use of the AFIT array in a second implantation experiment. At the conclusion of this thesis effort these details have now been addressed. Listed below are the major accomplishments and contributions of this thesis effort.

- An extensive literature review was conducted to determine if the proposed study was sound. This review showed that surface electrodes are useful for cortical research. Furthermore, the review implies that the AFIT multielectrode array is unique in that there were no other mention of a two dimensional array of surface electrodes in the literature.
- Further testing of the AFIT multielectrode array was conducted to determine if the device could meet the requirements gleaned from the current knowledge of cortical physiology. This testing showed that the device could be used to measure cortical signals which are at significantly lower amplitudes than levels previously tested.
- Relevant experiments were devised using both the knowledge of cortical physiology and the knowledge of the AFIT array's operational capabilities. Details of the experiments were analyzed to ensure that the experiments could be successfully performed. This process included surgical dry runs, which led to the identification of a potential problem with the previous design of the implantation package.

- The implantation package was redesigned to alleviate problems discovered during the surgical dry runs. The new design is both smaller and lighter than previous designs.
- The instrumentation setup needed to perform the experiments was designed, constructed, and fully tested. This design allows a much quicker evaluation of the data being recorded than the methods used in the first implantation.
- Finally, a detailed animal use protocol was developed. This protocol resulted in approval of the proposed AFIT study.
- Implantation of the device is only awaiting fabrication of additional chips.

4.2 Recommendations

4.2.1 Encapsulation and Packaging. Post processing of the AFIT array chips was an extremely time consuming task with many potential sources of errors. The application of polyimide was especially time intensive and several devices were ruined by various mishaps during these steps. It is possible that a commercial passivating process could be found which would decrease the time to process a device and which would also increase the yield.

Another possible alternative which should be pursued is the redesign of the device such that all active components are not interspersed with the electrodes. Application of polyimide would be much simpler with such a design. Furthermore, this design would be less susceptible to failure induced by the saline environment.

4.2.2 Design of Experiment. More promising experiments using the AFIT array will involve the use of trained primates. These experiments will be more complex than the current study. Therefore, if future implantations are planned, an effort to enlist the aid of a experienced primate researcher should be made. Additionally, the protocol approval process should be started as early as possible in any follow-on thesis efforts.

4.2.3 Design of the AFIT Array. There are several improvements which should be made to the AFIT array. Currently, the output of the device is very noisy. Part of this noise comes from the external clock signal. Unfortunately, it is undetermined how this clock signal is propagated to the device output. Future designs of the device should make all attempts to reduce the noise on the device output. Addition of a differential amplifier to the device could solve some of this noise problems. The device could be redesigned such that adjacent electrodes are selected to provide the two inputs to the differential amplifier. This design should eliminate all common sources of noise and should also allow the device to record signals with even lower amplitudes.

The current design restricts the use of negative voltage signals from being used for stimulation. This restriction could be removed by the use of a negative Vss input to control the transmission gates. With this change, a more complex stimulus signal could be used. An interesting experiment would be to 'replay' a gamma–burst type signal (which has both positive and negative peaks) as the stimulus to see if a cortex wide response could be elicited.

Appendix A. Biological Background

A.1 Neurons.

The fundamental building block of the brain is the neuron. Neurons are composed of three major parts: the cell body, which includes the cell nucleus; the dendrites, which are highly branched extensions used to receive in-coming signals; and the axon, which are unbranched extensions that transmit the signal from the cell body [13].

Information is transmitted by the generation of electrical impulses known as action potentials. These signals propagate down the cell's axon and are converted to chemical signals at the synapses, which are the contact points between neurons. The generation of action potentials is described by Fischbach [13]. In brief, action potentials result from the movement of positively charged ions, primarily sodium and potassium, across the cell membrane into the cell interior. At rest, a neuron's external membrane maintains an electrical potential difference of about -70 mV. When the cell is stimulated, the membrane's permeability to sodium increases, leading to an in-rush of positive charges. At some threshold potential, this in-rush causes a momentary reversal of the membrane potential which triggers an impulse. The sodium permeability declines after about one millisecond and the membrane potential is returned to its' resting value. Action potentials measure about 100 mV in amplitude and last about 1 millisecond. Physical restrictions limit action potential generation to about 200–300 per second average rate. Note that the action potential amplitude and duration is essentially invariant. Therefore, it is the frequency of the action potential that transmits the intensity of a neuron's output.

Action potentials cannot jump from one cell to another. Neurons transmit data to other nerve cells through the use of chemical neurotransmitters released at the synapses. These transmitters trigger a change in the membrane permeability. Some neurotransmitters, labeled excitatory, have a depolarizing effect on the target

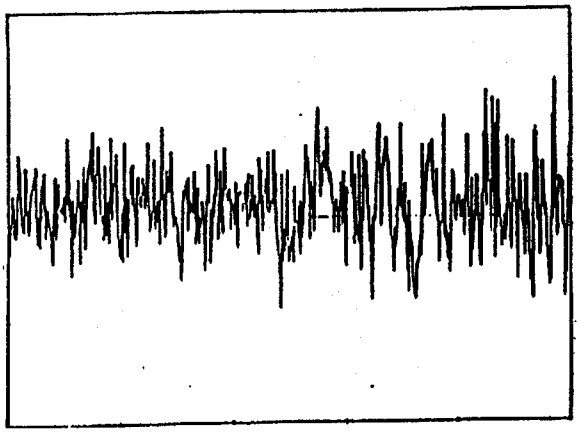


Figure 21. Electroencephalogram tracing [26].

neuron which brings the neuron closer to the action potential generation. Other neurotransmitters have an inhibitory effect which tends to stabilize the membrane at the resting potential [14]. Each synapse has a small effect on the cell. The neuron integrates up to 1,000 synaptic inputs to set the intensity of its' output [13]. It is believed that the electric field produced can be received by small electrodes resting on the surface of the cortical tissue. Consequently, an electrode would record the data processing activity of the neurons beneath it.

The electric field measurements taken from the surface of the brain are called electroencephalogram (EEG) tracings. A representative EEG is shown in Figure 21. The mean excitatory state of the group of neurons lying directly beneath the measuring electrode is reflected in each tracing. Increased excitement is indicated by rises in the traces. Similarly, dips in the tracings indicate diminished excitement caused by inhibition.

A.2 The Brain.

As mentioned earlier, the neuron is the fundamental building block of the brain. A human brain weighs only three to four pounds but contains about 100 billion neurons [13]. Visual, auditory and somesthetic information is processed in the outermost layer of mammalian brain, called the cortex. Figure 22 shows the

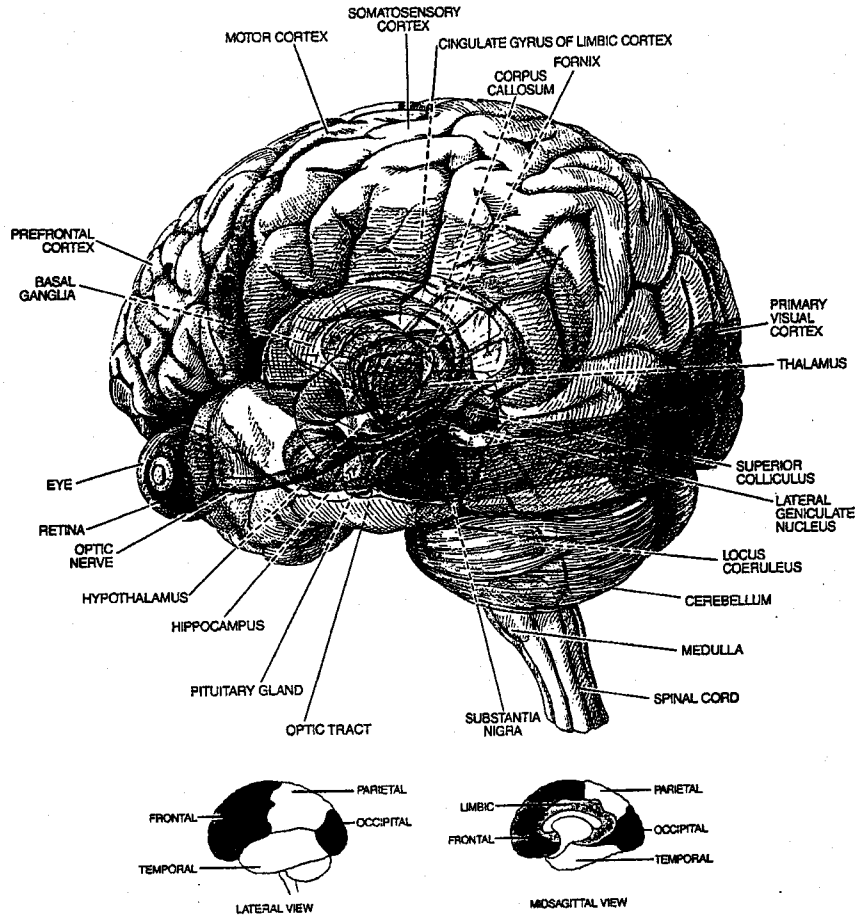


Figure 22. The human brain [13].

structure of the human brain. The cortex is divided into six layers of about 50 to 100 neurons thick. The layers are sequentially numbered from the outer surface of the cortex to the inner layer where it interfaces with the white matter (cerebral alba) of the brain, which is an area of very dense interconnections.

Anatomical research by Santiago Ramon y Cajal indicates that the flow of data in the cortex is primarily vertical. Furthermore, Vernon B. Mountcastle, working on the somatosensory cortex [30], and David H.Hubel and Torsten N. Wiesel, working on the visual cortex [20], observed that the cortex is grouped into cortical columns, which consist of several hundred to several thousand neurons functioning together

to perform a specific task. Therefore, these cortical columns, which Kabrisky termed "basic computing elements," are believed to be the smallest functional elements in the cortex [26]. These cortical elements, which extend through the thickness of the cortex, have been estimated to be as small as 50 μm in diameter in the human primary visual cortex to as large as 500 μm for a cat [20].

As the anatomical studies show, there is virtually no transmission of data *across* the cortical sheet. The cortical columns interact only slightly with neighboring columns and are accessed primarily through input/output axons running through the cerebral alba and between the cortex and the brain stem. Kabrisky has suggested that an analysis of the interconnecting matrix between cortical columns may provide new insight to the function of the brain [26]. Since an EEG gives the excitatory level of a group of neurons it is believed that it is the activity of the cortical columns which will be measured by a surface multielectrode array.

The cortex can also be subdivided into functional areas. These areas, such as areas for the numerous sensory inputs, motor-control, and associative areas have been well mapped through anatomical studies. The relative location of these areas can be seen in Figure 22. Note the location of the visual cortex, the area responsible for processing visual information, is the occipital lobe to the rear of the brain.

A.3 Visual Perception System.

Of particular interest is the area related to visual perception. As mentioned earlier, although a large amount of research has been performed on the visual perception system, the methods used by the visual cortex for processing visual information remain unknown. This research has yielded extensive knowledge about the interconnections between the retinae and primary visual cortex, also known as area V1. In brief, the rods and cones of the retina transform light into neural signals. These signals are connected to the primary visual cortex through a relay structure within the brain known as the lateral geniculate nucleus (see Figure 23). This connection

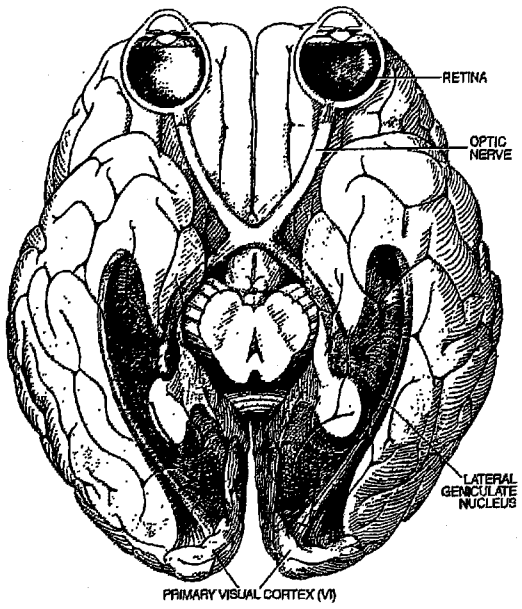


Figure 23. The visual pathway [41].

is made such that it is a precise mapping of the visual field onto V1 [41]. This mapping was verified in experiments in which laboratory monkeys were fed radioactive glucose and made to stare at a flickering pattern. Hypothesizing that a cell which is more active would use more of the tagged glucose, it was possible to determine what portion of the visual cortex was stimulated by comparing the tissue radioactivity. Figure 24 shows the pattern of brain activation caused by a "wagon-wheel" type visual stimulus pattern. Considering that the cortex is not flat there is an excellent correlation between the patterns of the stimulus and brain activation. The results of this experiment suggest that the visual pattern had indeed been mapped onto V1 [38].

It is also known that each point on the primary visual cortex connects to several other points on the visual cortex, identified as areas V2-V5. These areas are believed to be individually specialized to perform specific tasks [41]. Since the primary visual cortex is basically a mapping of the visual scene and there is limited horizontal transfer of signals, the pattern recognition process must occur in

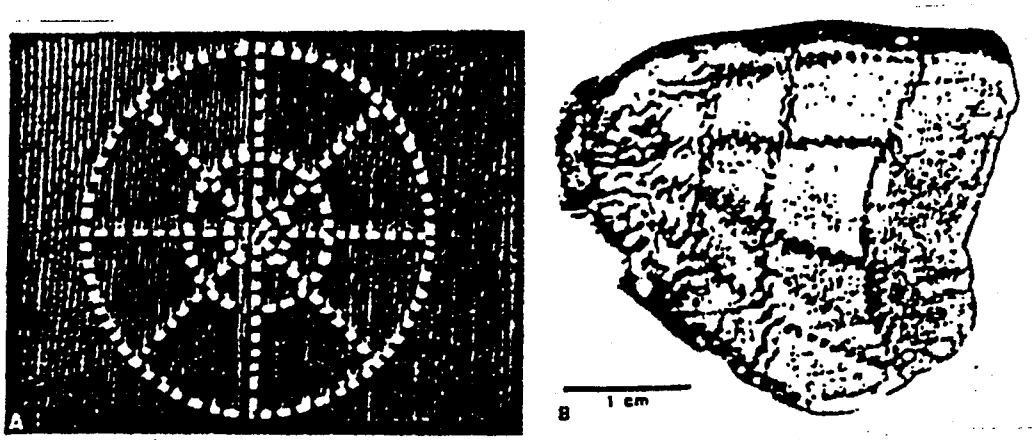


Figure 24. (A) Visual stimulus used in radioactive glucose experiment. (B) Pattern of brain activation produced by the visual stimulus shown in (A) [38].

the interconnections between the primary visual cortex and the secondary visual areas [26]. Nature has performed some 'what-if' type experiments related to the interconnections between the retina and associated areas of the brain in the form of lesions or strokes. Neurologists note that a lesion in the pathway to V1 created a blind spot whose position corresponded precisely to the location of the lesion in V1 [41]. This result supports the idea that the primary visual cortex contains a map of the external visual field.

Appendix B. Protocol

ANIMAL USE PROTOCOL COVER SHEET

1. Title: A Multielectrode, Multiplexed Silicon Cortical Electrode

2. Purpose of the Study:

To test *in vivo* the performance of a multielectrode, multiplexed cortical electrode. This 16 × 16 array (256 total) electrode system is designed to both measure naturally occurring distributed cortical activity and to insert two- dimensional data into the cortex. In the later mode it could serve as a visual prosthesis for blindness caused by lesions distal to the cortical mapping of visual data. The electric field measurements taken from the surface of the brain are called electroencephalogram (EEG) tracings which represent the mean excitatory state of the group of neurons lying directly beneath the measuring electrode. The performance of the multielectrode array will be assessed by the ability of the device to record EEG data.

3. DoD Relevancy:

Animal visual systems have certain capabilities which have direct military application. Primates especially have visual systems with high foveal acuity, color discrimination, and gestalt-based form perception. If such capabilities could be duplicated by machine, they would have immediate direct application in the automatic target acquisition and terminal guidance phases of smart missile systems. Current "smart" weapons still require a human operator to find the target and set up a laser based terminal guidance system. Alternatively, the U.S. Army has developed a series of TV-guided, anti-tank missiles in which a TV picture from the missile is transmitted to a human guidance operator who actually "flies" the missile by remote control.

No reliable automatic target locator or flight guidance system based on visual form has ever been put into operational service even though research on this so called "pattern recognition" problem has been vigorously pursued for about forty years. It is clear that there are some fundamental capabilities immanent in mammalian visual systems that we simply do not understand; if these naturally evolved techniques could be discovered from properly instrumented animal visual systems, it might be possible given the current advanced state of electronic fabrication capabilities, to make a fundamental breakthrough in weapon guidance systems.

4. Type and # of Animals:

Species: Macaca mulatta
Sex: Male
Weight: 12 KG
Age: 12 to 15 years
Total number required: 1

Species: Mustela putorius
Sex: Male
Weight: 1 KG
Age: 12 months
Total number required: 3

5. Disposition of Animals:

The study is designed to allow for the animal to return to the colony following surgical removal of the chip. In the event the health of the animal deteriorates or for some other reason, euthanasia is indicated, the animal will be administered sodium pentobarbital intravenously to effect.

6. Principal Investigator/Organization/Extension:

Steven K. Rogers, AFIT/ENG, 255- 6565, x4284
Matthew Kabrisky, AFIT/ENG, 255-9267.

CORTICAL ELECTRODE ANIMAL PROTOCOL

1. Title: A Multielectrode, Multiplexed Silicon Cortical Electrode

2. Project/Task/Work Unit:

3. Principal Investigator:

Steven K. Rogers, AFIT/ENG
Matthew Kabrisky, AFIT/ENG

4. Associate or Co-Investigators: James R. Cooper, AL/OEVM

5. Scientific Objective:

To test *in vivo* the performance of a multielectrode, multiplexed cortical electrode. This 16 × 16 array (256 total) electrode system is designed to both measure naturally occurring distributed cortical activity and to insert two- dimensional data into the cortex. In the later mode it could serve as a visual prosthesis for blindness caused by lesions distal to the cortical mapping of visual data. The electric field measurements taken from the surface of the brain are called electroencephalogram (EEG) tracings which represent the mean excitatory state of the group of neurons lying directly beneath the measuring electrode. The performance of the multielectrode array will be assessed by the ability of the device to record EEG data.

6. Military Relevance:

Animal visual systems have certain capabilities which have direct military application. Primates especially have visual systems with high foveal acuity, color discrimination, and gestalt-based form perception. If such capabilities could be duplicated by machine, they would have immediate direct application in the automatic target acquisition and terminal guidance phases of smart missile systems. Current "smart" weapons still require a human operator to find the target and set up a laser based terminal guidance system. Alternatively, the US Army has developed a series of TV-guided anti-tank missiles in which a TV picture from the missile is transmitted to a human guidance operator who actually "flies" the missile by remote control.

No reliable automatic target locator or flight guidance system based on visual form has ever been put into operational service even though research on this so called "pattern recognition" problem has been vigorously pursued for about forty years. It is clear that there are some fundamental capabilities immanent in mammalian visual systems that we simply do not understand; if these naturally evolved techniques could be discovered from properly instrumented animal visual systems, it might be possible given the current advanced state of electronic fabrication capabilities, to make a fundamental breakthrough in weapon guidance systems.

7. Technical Background:

The AFIT multielectrode array design was based on several key concepts:

- Information is transmitted in a neuron by the generation of electrical impulses known as action potentials.
- The electric field produced by the activity of neurons can be received by an electrode resting on the surface of the cortical tissue.
- The neurons in the cortex are grouped into cortical columns, which consist of several hundred to several thousand neurons functioning together to perform a given task. These cortical columns are believed to be the smallest functional elements in the cortex.
- There is virtually no transmission of data *across* the cortical sheet. The cortical columns are accessed primarily through input/output axons running through the cerebral alba and between the cortex and the brain stem.
- Analysis of the interconnecting matrix between cortical columns may provide new insight to the function of the brain.

Research similar to that being proposed by AFIT relies on the use of macroelectrodes arranged as arrays of either intracortical probes or surface probes. Intracortical probes are designed to penetrate the cortical surface in order to record the response of (or stimulate) small groups of cortical neurons completely in the interior of the cortical column. Surface electrodes, on the other hand, are used to record or stimulate the *group* of neurons directly beneath the electrode. The electric field measurements taken from the brain by macroelectrodes are called electroencephalogram (EEG) tracings. An EEG represents the mean excitatory state of the group of neurons lying in close proximity to the measuring electrode. Therefore, it is reasonable to expect a surface electrode to record the response of the neurons directly below. Whereas the intracortical probes allow studies primarily in the interior of the cortical sheet.

Properties of the intracortical probe concept emphasize the importance of getting more intimate connection to the interior of the cortical column system. They seem to overlook the inevitable damage which these electrodes cause. But the fact that the columns behave as unitary devices leads us to assume that individual columns can be effectively stimulated from the cortical surface ends of the columns as in fact has been done for decades. Therefore, the AFIT electrode is placed on the cortical surface where it will produce little or no trauma to the cortex beneath it and where it will still have access to the columns for recording and stimulation purposes.

Early research using arrays of surface electrodes relied primarily on the use of bundles of very fine wires placed on the surface of the cortex. In 1966 DeMott reported on the use of a 400 electrode array made up of 400 closely spaced (0.25 mm center-to-center) wires. DeMott's results obtained from a variety of animals, demonstrated substantial differences in the behavior of virtually adjacent cortical areas. This behavior is consistent with the existence of distinct functional cortical columns described by Mountcastle (1957) and Hubel and Wiesel (1962).

In 1968 Brindley published the description of a multielectrode visual prosthesis which he had implanted in the primary visual cortex of a blind human volunteer. This electrode system used 80 separate wires arranged in an approximate 8×10 array; each wire was brought out of the subjectës skull and connected to a multi-pin electric connector. Electrical stimulation of any one of the electrodes produced the subjective experience of "seeing" a point of light in a specific location of the subject's visual field. Brindley termed these "phosphenes" and considered them as stimulatable pixels. Thus by stimulating them in appropriate combinations he could transmit crude geometrical images to the visual system of a blind person. Needless-to-say, electrical stimulation of the brain causes no physical discomfort to the subject since there are no pain receptors in the cerebral cortex.

In 1974, Dobelle reported replication of this procedure and produced a demonstration movie showing a blind subject reading Braille letters that had been inserted into his visual cortex by stimulating appropriate sets of electrodes. The subject in Dobelle's experiment had been blind for 10 years and was able to read Braille at 30 letters a minute using a 64 electrode array for phosphene generation.

Semiconductor technology overcomes many of the problems associated with using wire bundle electrodes. Wire electrodes suffered from poor control over their physical and material characteristics, resulting in a lot of variation in measurements. Also, the number of electrode sites was limited by the amount of wires which could be passed through the scalp. With the current semiconductor technology, multiplexing of the data is easily performed, greatly reducing the number of connections through the scalp. A few of the devices made possible by semiconductor fabrication techniques are discussed in the following paragraphs.

Researchers at the University of Michigan have designed a multichannel multiplexed intracortical probe. This device,which resembles a key, is designed to be inserted into the cortical tissue. The probe is typically 15 μm in thickness with a shank width as narrow as 20 μm. Multiple electrode sites are exposed along the length of the shank. The electrode sites are multiplexed together to allow individual electrodes to be used for recording and or stimulation. By attaching multiple probes in a precisely controlled pattern to an orthogonal platform, a three- dimensional array of electrodes is possible. Such a three-dimensional device can be used to monitor neural activity throughout a volume of cortical tissue. However, use of a intracortical probe does traumatize the tissue. Bleeding was observed during experiments using gerbils. Additionally, probe electrodes were found to have been coated with biological material which may affect the recording capabilities of the electrodes.

Jones, et al. at the University of Utah have also developed a three-dimensional intracortical electrode array. This device consists of a micro machined array of 100 silicon "needles" on a $4.2 \times .2 \times 0.12$ mm thick substrate. The tip of each sharpened needle is coated with platinum and functions as the electrode site. The device is being designed for sensory restoration via intracortical electrical stimulation of a sensory

cortex. Experimental implantation of the device has been performed on cats with evidence of some intracortical bleeding due to blood vessel damage being observed. Also, only a few electrodes were able to be accessed with their current technique; a demultiplexer chip to allow access to all electrodes was still in development.

Dagnelie, et al. recorded visually evoked potentials (VEPs) in an alert rhesus monkey using an array of 35 electrodes. The electrodes were organized into five bundles of seven wires each, and were implanted on the striate and peristriate visual cortex. The research was performed as a detailed study of cortical mapping but also demonstrated the usefulness of surface measurements in the study of cortical processing.

Bartlett and Doty investigated the ability of monkeys to detect microstimulation of the striate cortex. In their experiments, an array of twelve electrodes were implanted within the representation of central vision in the visual cortex of a monkey. The animal was then trained to respond to the application of 0.2 ms electrical pulses at 50 Hz to its striate cortex. The purpose of this study was to measure the threshold for the detection of stimulus. The threshold for detection of the stimulus ranged from 50 to 250 μA, depending on the depth of penetration into the cortex. Bartlett and Doty did not test the animal's response to any visual stimulus.

A lot is known about how the brain initially analyzes sensory messages. Yet, how the brain combines sensory messages with past experience for recognition is still not known. Freeman believes that a macroscopic view must be adopted in order to understand perception. His research suggests that perception depends not on the action of individual neurons, but on the simultaneous, cooperative activity of millions of neurons spread throughout the cortex. Freeman's studies were concentrated on the neurons of the olfactory system. Action potentials from the receptor neurons in the nasal passages propagate to an area of the cortex known as the olfactory bulb. From there, new signals are sent to many other parts of the brain. Note that this is very similar to the way visual signals are received by the primary visual cortex. EEG data was collected simultaneously from 60 to 64 electrodes 0.5 mm apart attached to the surface of the olfactory bulb of trained rabbits.

In Freeman's experiments, a "burst" of oscillations could be seen in each EEG tracing when an animal inhaled a familiar scent. All the tracings from the electrode array were suddenly more regular for a few cycles, until the animal exhaled. Also the tracings often had a higher amplitude($\sim$100 μV) and frequency (from 20 to 90 Hz). These bursts are labeled as gamma waves denoting the frequency band of the observed oscillations. Evidence of a collective behavior was shown by the presence of a common waveform, or carrier wave, present in each tracing in the sets of burst recordings. Freeman found that it is not the shape of the carrier wave that identifies an odorant. In fact the wave changed every time the animal inhaled. However, when the average amplitude of each electrode's carrier wave was plotted on a grid representing the olfactory bulb surface, a specific amplitude pattern emerged. As

long as the animals training was not changed the same map resulted for a particular odorant, even though the carrier wave was different for each sniff.

Freeman found other parts of the brain may exhibit the same chaotic behavior seen in the olfactory system. In fact, he has documented gamma bursts across large cortical regions involved in the recognition of visual images. His research suggests that familiar visual stimuli are also associated with specific amplitude maps of common carrier waves. Freeman predicts that when viewing a drawing in which foreground and background is ambiguous, such that perception alternates between the two images, the amplitude maps will be found to alternate as well. Similar oscillatory responses were discovered in the cortex of cats and the macaque monkey by Gray and Engel.

It seems likely that interconnection systems between coupled arrays of cortex will be multichannel arrays of axial trunks interconnecting two dimensional arrays of cortical elements. Therefore, the two dimensional AFIT array of electrodes should be the best possible arrangement of detection and stimulation to couple to these systems.

Twelve years have past since the implantation of the first AFIT multielectrode array. This device, which consisted of sixteen multiplexed electrodes arranged in a four-by-four array, was implanted on the visual cortex of a laboratory beagle in 1982. The device functioned for fifteen days before being removed and the subject "Ricky" recovered with no apparent side effects. This experiment validated the concept of using multielectrode arrays for neural research; however, it also identified several improvements that were necessary. The improvements included the need for more electrodes, smaller electrodes to better match cortical columns, better fabrication processes to improve the electrical characteristics, and an improved surgical and implantation procedure. In the years since then, several thesis efforts have been made to improve the device design and to solve the problems encountered with the first implementation.

The current design of the AFIT multielectrode array is described in detail by Rob Reid in his 1993 thesis . This device consists of 256 electrodes laid out in a 16×16 array. A photograph of this device is shown in Figure 1. Each electrode can be enabled or disabled individually. An on-chip counter circuit selects each electrode in sequence as controlled by an external clock input. The electrodes are 160×160 μm with a center-to-center spacing of 250 μm. This device was tested in a saline environment simulating the environment of the brain, and found to function correctly.

From the physiological studies, the well known anatomical data, and the research on human volunteers, it is clear that it is possible to insert subjectively perceivable image-like stimuli into mammalian visual systems. Therefore, it is reasonable to assume that if an animal were trained to respond to a specific visual stimuli by, say, pressing a lever to get a food reward, then the insertion of such a

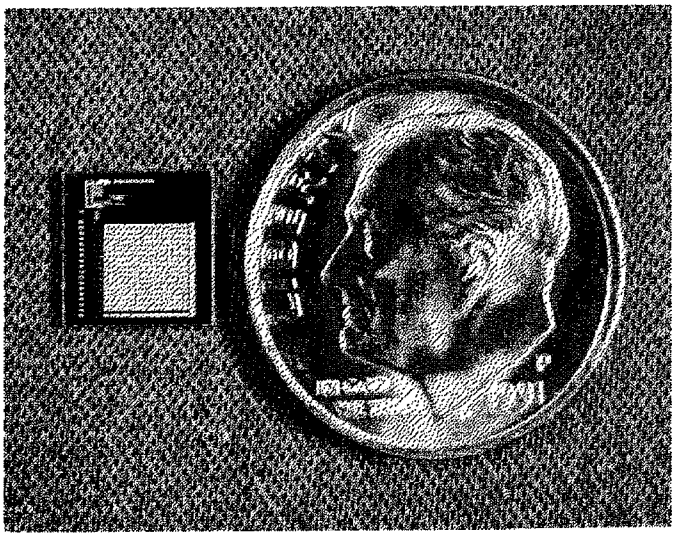


Figure 1. The AFIT multielectrode array.

visual form directly into its primary visual cortex by electrical stimulus should result in the trained response. Such an experimental result would verify the integrity of the electronic and physiological systems as well as the experimental hypothesis that direct stimulation of animal visual cortex could duplicate the results obtained in human volunteer subjects.

Success at this stage suggests an even more interesting extension of the procedure. While damage to the human primary visual cortex produces only blind spots in the visual field, damage in secondary visual processing areas of the cortex produces categorical visual deficits lumped under the general term: "agnosias". One of these, as an example, prosopagnosia is the complete inability to recognize human faces, even oneës own, while retaining otherwise normal visual perception. When a brain so damaged is recovered after death, the only obvious damage is the destruction of a small piece of cerebral cortex in a secondary visual processing area. This leads to postulating a system where visual data are first mapped to primary visual cortex where no significant form analysis (or "perception") occurs since damage there only causes circumscribed blind spots in the visual field. Following this mapping, the visual data are transferred by cortico-cortical tracts in the cerebral alba to secondary visual areas in the cortex where the elegant analysis we call perception actually occurs. Damage to these secondary regions causes catastrophic perceptual disease. It would be of great interest to monitor primary and secondary visual areas simultaneously in an attempt to gather data while the brain is actually performing perception.

Furthermore, it is reasonable to expect that a monkey trained to respond to a certain stimulus such as a light bar at a particular orientation could be used to demonstrate that the representation of a stimulus is manifested by a particular gamma burst. If the response to the stimulus was recorded and "played back"

through a stimulating electrode, the monkey should respond as trained just as if the actual visual stimulus was given. Success of such an experiment would depend on the ability to elicit a cortex-wide burst and also serendipitous placement of the electrodes.

8. Experimental Design:

This experiment will consist of two phases. Phase one will involve the use of ferrets to verify that the AFIT array is capable of recording EEG data from the cortex. Other investigators have found that the ferret is well suited for studies of the visual system (Jackson and Hickey, 1985). Phase two will involve the use of a Rhesus monkey. The array will be used in both species for recording and stimulation. Success of these two phases will be determined by the ability of the device to record EEG and VER data.

a. The anesthetic regimen and surgical approach will be the same in both species. Prior to surgery, the animal will be pre-anesthetized with Ketamine HCL (15 mg/kg) and Atrophine (0.04 mg/kg). Once anesthetized, the head will be shaved and prepared for sterile surgery. Surgical anesthesia will be induced using a 3-5% concentration of Isofluorane in combination with a 80/20 mixture of oxygen and nitrous oxide. The animals heart rate, respiratory rate, and blood pressure will be monitored during the surgical procedure.

The surgical procedure will consist of incising the skin and subcutaneous tissues over the visual cortex. The cranial muscles will then be bluntly dissected exposing the skull. A trephine will be used to create a circular opening in the skull. The device, shown in Figure 2, will be mounted in the circular opening in the skull and coupled to an external connector by a thin cable. The chip and associated mounting media will then be bonded to the skull using a polymalient glass ionomer cement. Three stainless steel bone screws will be tapered into the skull at approximately equal distances around the circumference of the trephine opening for use in anchoring the external connector. Liquid dental acrylic will then be poured over the exposed surface of the skull and the anchoring screws forming a skull cap which will be used to provide support for the external connector.

Once the dental acrylic has hardened, the skin and subcutaneous tissues will be apposed to the surface of the skull cap using a pruse string suture pattern. The animal will then be allowed to recover from anesthesia. An animal caretaker will monitor the animal continuously post surgically until it is able to maintain itself in an upright sitting position.

Seven days following surgery EEG data and Visual Evoked Response (VER) data will be collected from the animal in it's normal awake state. This process will require the animals to be restrained for approximately 10 minutes each day during the recording period. The ferrets will be restrained by simply having a technician hold the animal on their lap. The non-human primate will be restrained by placing the animal in a commercially available restraint chair which has been specifically

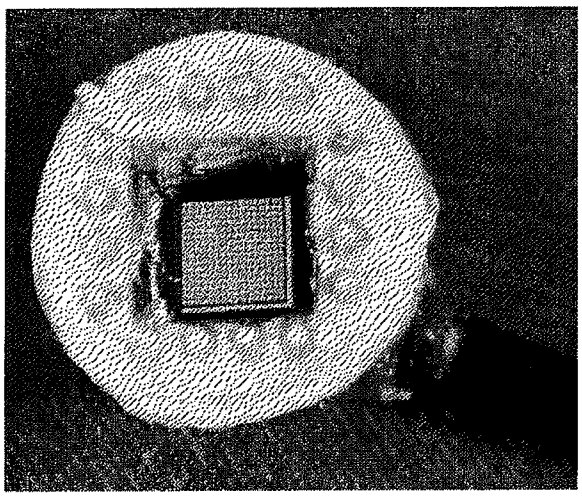


Figure 2. Mounted AFIT Array.

designed for this species. The monkey will be adapted to the chair during three weeks of training conducted prior to surgery. It is essential that the animals be comfortable and free of distress during the recording period not only for humane reasons but also to assure the validity and reproducibility of the resulting data.

The animal will be monitored as long as the chip is functioning. At the time the chip becomes non-operable it will be removed using identical surgical procedures as were employed during installation. A stainless steel plate will then be placed over the defect in the skull and secured in place using bone screws. The skin incision will be closed using subcutaneous sutures.

b. Statistics: This study will involve only one animal, therefore, no statistical tests will be performed.

9. Literature Review:

A literature search was conducted using Medline, Agricola, and Biological abstracts data bases to assure this study was not a duplication of one already reported in the literature. Based on the results of this search it is determined that this study is unique. Likewise, a DTIC search (search #DKJ35A) conducted on 18 April 1994 revealed no indication that this work has been accomplished previously.

10. Animal Utilization:

Species: Macaca mulatta	Species: Mustela putorius
Sex: Male	Sex: Male
Weight: 12 KG	Weight: 1 KG
Age: 12 to 15 years	Age: 12 months
Total number required: 1	Total number required: 3

a. Three ferrets are requested in phase one of the study in the event that initial testing reveals the chip requires minor modification and/or additional evaluation

prior to its implantation into a non-human primate. If the chip appears to be functioning as expected following implantation into the first or second animal, phase one testing will be halted.

b. Use of Alternatives: The use of alternatives such as computer simulations or in vitro techniques were considered. However, the intent of this research is to study the neural electrical activity of the cerebral cortex as it relates to the processing of visual images within the mammalian brain. No in vitro or mechanical system yet developed can approach the level of sophistication required for this study.

c. Rationale: Ferrets were selected for phase-one of the study in an attempt to use the lowest phylogenetic species of animal possible to obtain preliminary data on the operational capabilities of the chip. Primary requirements were that the animal must have a well defined visual cortex and a brain of sufficient size to mount the chip and associated connecting devie. Prior research by other authors supports the use of ferrets in visual system research (Jackson and Hickey, 1985; Wen and Shek, 1985).

d. Primate Justification:

(1) There are four primary reasons the non human primate was selected for this study. First, the study requires a species with a brain in which the visual cortex is surgically accessible and of sufficient size to allow placement of a 0.5 $\times$ 0.5 cm chip. Second, later stages of this research will require a species which can be readily trained to respond to visual images originating either through the normal optic pathways or via electrical impulses delivered directly to the visual cortex. Third, use of the non human primate allows more efficient use of data already collected in the highly sophisticated human visual system. Fourth, data obtained from this study will be directly applicable to the human species.

(2) The study is not designed to require euthanasia of the animal.

e. Post Experimental Disposition: Ferrets will be added to an existing ferret colony within the facility. Likewise, the non-human primate will be returned to the primate colony following surgical removal of the chip. In the event the health of the animal deteriorates or for some other reason euthanasia is indicated, sodium pentobarbital will be administered intravenously to the animal in an amount sufficient to elicit a painless death. If euthanasia is performed for the non-human primate due to poor health, it is felt that the body parts would not be useful. On the other hand, if euthanasia is prescribed for reasons other than deteriorating health, an attempt will be made to distribute organs and other body parts to other investigators within the laboratory who may have a requirement for these tissues.

11. Relief of Pain or Distress:

a. This study is classified as category B in the Wright-Patterson AFB "Pain and Distress Classification System." "The research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthet-

ics/analgesics." The surgical procedures involved in this study would cause pain to the subject animal if not relieved with anesthetic drugs. Human patients who have had similar devices installed within their skulls to record and provide electrical stimulation to their visual cortex have not indicated the procedure was painful.

(1) The animal will be pre anesthetized with Ketamine HCL (15 mg/kg) and atrophine (0.04 mg/kg). Surgical anesthesia will be provided using a 3-5% concentration of Isoflurane superimposed on a 80/20 mixture of oxygen and nitrous oxide. Post-operative analgesia will be provide by administering Buprenorphine (0.01 mg/kg) twice daily by intramuscular injection for 4 days or longer if deemed necessary.

(2) NA

(3) The following data bases were searched in an attempt to determine a less painful or distressful means of conducting this study: Agricola, Biological abstracts and Medline. No less painful or distressful means of conducting the study were found.

(4) Death is not an endpoint in this study. Should the animal become debilitated, loose in excess of 10% of its body weight, refuse to eat or drink, or in some other way indicate that it was in non relievable pain, it will be humanely euthanized.

(5) Dr. John Latandresse DVM/PhD was consulted in the design of this study.

12. Personnel Training:

The surgical procedure will be performed by a veterinarian who has had previous experience in the placement and removal of cortical electrodes and skull caps. All animal manipulations, and post operative care procedures will be performed by personnel certified by the American Association for Laboratory Animal Science (AALAS) at the technologist level.

13. References:

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14. Hazardous Agents: This study will not employ the use of hazardous agents.

15. AFSC Form 3530: Attached

Appendix C. AFIT Array Post-processing Steps

C.1 Materials

Photo-Resist Spinner
Headway Research, Inc.
Model 1-PM101D-R790

Sputtering System
Denton DV-602RF

Acetone
Ashland Chemical Company
Columbus, OH 43216

Adhesion Promoter, Hexamethyl Disilazine (HMDS)
Mallinckrodt, Inc.
Paris, Kentucky 40361

Butyl Acetate
Mallinckrodt, Inc.
Paris, Kentucky 40361

Chlorobenzene
Mallinckrodt, Inc.
Paris, Kentucky 40361

Negative Photo-resist
Waycoat HR-200
Olin Hunt Specialty Products
West Paterson, New Jersey 07424

Positive Photo-resist
Microposit 1350J
Shipley
2300 Washington Street
Newton, MA. 02162-0469

Positive Photo-resist developer
351
KTI Chemical, Inc.
1170 Sonora Court
Sunnyvale, CA. 94086

Polyimide PI-2555
Pyralin Polyimide Resin
Dupont Company
Semiconductop materials Group
Wilmington, DE. 19898

TO-8 Header
Phillips Technology
Airpax Protection Group
807 Woods Road
PO Box 520
Cambridge, MD 21613-0520

VM651 Adhesion Promoter
Pyralin Polyimide Coatings
Dupont Company
Semiconductop materials Group
Wilmington, DE. 19898

C.2 Standard Clean

1. Mechanically agitate chips in an acetone bath for 30 seconds.
2. Immerse chips in a bath of methanol for 1 minute.
3. Blow chips dry with N_2.
4. Bake chips at 110° C for 15 minutes.

NOTE: Do not clean the chips with an ultrasonic bath. This process could cause visually undetectable damage to the device.

C.3 Metalization Process

1. Clean with standard clean process.
2. Remove from oven and allow to cool.
3. Blow chips with N_2 to remove surface dust.
4. Apply adhesion promoter (HMDS)

- Puddle HMDS onto the chip. Allow it to spread over the entire chip and coat the edges.
- Allow to sit for 5 seconds to ensure good coverage of the chip.
- Spin at 5000 RPM for 45 seconds.

5. Apply positive photo-resist (Microposit 1350J)

- Puddle 1350J onto the chip. Allow it to spread over the entire chip and coat the edges.
- Allow to sit for 5 seconds to ensure good coverage of the chip.
- Spin at 5000 RPM for 45 seconds.

6. Pre-bake at 70° C for 20 minutes.

7. Align/Expose for 60-65 seconds.

8. Immerse in chlorobenzene for 2 minutes.

9. Bake at 90° C for 15 minutes.

10. Develop photo-resist.

- Spin at 1000 RPM
- Spray with 351:deionized water (DIW) 1:3 for 45 seconds.
- Spray with DIW for 30 seconds.
- Spin dry at 5000 RPM for 45 seconds.

11. Examine pattern. If further development is necessary, develop for an addition time.

12. Post-Bake at 90° C for 15 minutes.

13. Sputter Ti for 25 minutes with forward power of 200 W (Approx. 300 Å).

14. Sputter Ir for 75 minutes with forward power of 150 W (Approx. 3000 Å).

15. Immerse in acetone bath. Lightly scrub with a cotton swab to ensure all undesired metal is lifted off of the chip.

C.4 Polyimide Application Process

1. Clean chips with the standard clean process.

3. Remove from oven and allow to cool.

4. Blow with N_2 - removes surface dust

5. Apply adhesion promoter (VM651): (Note the VM651 must be mixed in a solution of 190 ml methanol, 10 ml DIW, and 1 drop of VM-651, at least 12 hours prior to use. The mixed solution should not be used after 20 days.)

- Puddle VM651 onto the chip. Allow it to spread over the entire chip and coat the edges.
- Allow to sit for 5 seconds to ensure good coverage of the chip.
- Spin at 5000 RPM for 45 seconds.

6. Apply polyimide (PI-2555):

- Puddle PI-2555 onto the chip so that it flows over all edges.
- Allow to sit for 5 seconds to ensure good coverage of the chip.
- Spin at 4000 RPM for 45 second (Approx. 25 μm).

7. Pre-bake at 70° C for 15 minutes. - Dries the polyimide without curing.

8. Remove from oven and allow to cool.

9. Apply negative photo-resist (Waycoat HR-200):

- Puddle onto the chip allowing it to cover entire chip and sides.
- Spin at 5000 RPM for 45 seconds.

10. Pre-bake photo-resist at 70° C for 25 minutes.

11. Remove from oven and allow chips to cool.

12. Blow clean with N_2

13. Align/Expose for 4.0 minutes.

14. Develop photo-resist:

- Spin at 500 RPM
- While spinning spray with Xylene for 30 seconds.
- Spray with butyl acetate for 30 seconds.
- Spin dry at 5000 RPM for 30 seconds.

15. Examine pattern. If the pattern is not good, then polyimide and photo-resist must be stripped and the process repeated.

16. Etch the polyimide.

- Spin at 1000 RPM
- Spray with 351:DIW (1:5) for 3.5-7 seconds depending on which coating this is.
- Spray with DIW for 30 seconds.
- Spin dry at 5000 RPM for 30 seconds.

17. Examine to ensure the etch is complete. Etch for additional time if it is required.

18. Final cure at 180° C for 4 hours. Complete cure of the polyimide.

19. Repeat steps 1–18 for second and third layers of polyimide.

C.5 Packaging

1. Mount the chip on a header package and wire-bond the connections in accordance to Figure 10. (This step was performed by Larry Callahan at the Avionics Directorate of Wright Laboratories or Captain John Comtois, an AFIT PhD student.)
2. Apply a coat of polyimide (PI-2555) as follows:
   - Allow polyimide to reach room temperature prior to use.
   - Using a toothpick, apply polyimide over the wire-bonded connections being careful not to cover any of the electrode array or reference electrode.
3. To avoid expansion and contraction of the polyimide layer which could break wire bonds, the polyimide is cured using gradual changes of temperature as listed below:

Temperature (° C)	*Time (min.)*
70	30
85	30
105	30
130	30
165	360

NOTE: Allow packaged devices to cool slowly by turning off the heat for the oven and allowing it to cool to room temperature prior to opening the door.

4. Repeat steps 2 and 3 for a second coat of polyimide.
5. Apply a thin layer of silicon sealant over the wire-bonded connections and allow to dry.
6. Clip unused pins from the back of the header level to the surface.
7. Clip remaining wires to an approximately 3mm in length.
8. Solder wires to the connections in accordance with Table 2 and Figure 10.
9. Apply a thin layer of silicon sealant to the soldered connections and allow to dry.
10. Carefully bend the pins over towards the middle of the header to reduce the overall width of the implantation package.
10. Group the wires into a bundle using heat shrink tubing. Note: Cable lengths for the ferret and rhesus monkey are approximately 8 and 12 inches respectively.
11. Apply a final coat of silicon sealant to the back of the header to further insulate and secure the wire connections.

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Vita

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13. ABSTRACT *(Maximum 200 words)*

A two-dimensional, multiplexed array of 256 electrodes (16 × 16) has been fabricated using conventional CMOS fabrication techniques. The size and spacing of the electrodes (160 × 160 μm squares with center-to-center spacing of 250 μm) approximate that of the cortical columns. The device is mounted on a small, biocompatible package and a protocol to implant this package on the visual cortex of a ferret and a Rhesus monkey was developed and approved. An instrumentation setup to record the data from the device is described as well as the methods used to demultiplex the data. Testing of the device in a simulated cortical environment indicates that the device is capable of recording signals with amplitudes as low as 20 μV. This capability is significant as the level of the cortical signal ranges from 20–500 μV. An experiment is envisioned in which the primary and secondary visual areas of a mammal are monitored simultaneously in an attempt to gather data while the brain is actually performing perception. This type of study will provide insight to the nature of the organization of the cortico-cortical connectivity matrix and its relation to the function of the cortical column system.

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